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CHAPTER**17****Carrot Motley Dwarf Virus Complex**

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ABSTRACT

The carrot motley dwarf (CMD) disease is a complex of a luteovirus, an umbravirus and an infectious RNA that causes reddening, leaf chlorosis and stunting on *umbelliferae* such as carrot and parsley. The luteovirus has been identified as *Carrot red leaf virus* (CtRLV), which has recently been assigned to the genus *Polerovirus* within the *Luteoviridae* family. CtRLV acts as a helper virus for aphid transmission of the other components of the CMD virus complex. The umbravirus is either *Carrot mottle virus* (CMoV), which is associated with the CMD complex in Great Britain, Belgium and Morocco or *Carrot mottle mimic virus* (CMoMV), which is associated with the complex in Australia, New Zealand and California. For aphid transmission, the umbravirus genome is transcapsidated in the virions of CtRLV. In the field, CMoV and CMoMV probably only occur in mixed infections with CtRLV. The third partner in the disease complex is Carrot red leaf associated RNA (CtRLVaRNA), a 2.8 kb RNA which is very similar to the *Beet western yellows virus* ST9-associated RNA. The CMD virus complex is transmitted by the willow-carrot aphid, *Cavariella aegopodii* in a persistent, nonpropagative way. The aphid overwinters in the egg stage on its primary host, the willow tree, which is immune to CMD. First generation aphids on the willows are non-viruliferous and acquire the CMD virus complex from umbelliferous weeds or other umbelliferous crops. Detection of the CMD complex in plants or viruliferous aphids is based on a reverse-transcription PCR method using pairs of redundant umbravirus and luteovirus primers and a pair of specific CtRLVaRNA primers. Control of CMD is based on cultural methods aimed at reducing sources of CMD and its aphid vector or chemical control of *C. aegopodii*. Some resistance to CMD is available in certain carrot cultivars, but resistant parsley cultivars have not been reported.

Key Words: Carrot motley dwarf virus complex, luteovirus, umbravirus, infectious RNA, transmission, control

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1. INTRODUCTION

The carrot motley dwarf (CMD) disease was first described by Stubbs (1948) in Australia, where it caused serious damage to carrot (*Daucus carota* L.) production. Since then, the disease has been reported in Japan (Komuro and Yamashita, 1956), the USA (Stubbs, 1956), Canada (Murant, 1975), Europe (Watson, 1960; Heinze, 1968; Van Dijk and Bos, 1985), Israel (Marco, 1993) and Brazil (Figueira, 1995). Initially, CMD was thought to be associated with a single virus, named *Carrot motley dwarf virus* (Stubbs, 1948). Watson and Serjeant (1964) however, demonstrated that a complex of two single stranded RNA viruses, *Carrot red leaf virus* (CtRLV) and *Carrot mottle umbravirus* (CmoV), are the causal agents of the CMD disease. More recently, it has become clear that more than two viruses can be involved in the aetiology of the CMD virus complex. Gibbs *et al.* (1996b) found that co-infections of CtRLV and another virus, *Carrot mottle mimic umbravirus* (CmoMV), were associated with CMD in certain parts of the world. In 1998, an infectious RNA, named CtRLV-associated RNA (CtRLVaRNA) was identified as a third component of the Californian CMD virus complex (Watson *et al.*, 1998). Since then, CtRLVaRNA has also been reported in Belgium to be part of the CMD virus complex infecting parsley (Vercruysse *et al.*, 2000a) and in the UK as part of the CMD virus complex infecting carrot (Morton *et al.*, 2003). The CMD virus complex is transmitted by the willow-carrot aphid, *Cavariella aegopodii* Scopoli (Homoptera, Aphididae) in a persistent, nonpropagative way (Stubbs, 1948; Elnagar and Murant, 1978).

2. THE VIRUSES CAUSING CARROT MOTLEY DWARF DISEASE

2.1 Carrot red leaf virus

Carrot red leaf virus was first described from carrot in Great Britain (Watson *et al.*, 1964). CtRLV is a member of the *Luteoviridae* and has icosahedral virus particles (25 nm in diameter) that are non-enveloped (Waterhouse and Murant, 1981). The genome consists of a monopartite single-stranded positive sense RNA molecule of about 5.6 kb. Typical for luteoviruses, CtRLV has a narrow host range (certain members of the *Umbelliferae*), is obligately transmitted by aphids in a persistent way and not by sap transmission, and has a high degree of vector specificity. Virions are confined to phloem tissue and occur in very low concentrations in the plant (Miller, 1994; Randles and Rathjen, 1995). The virus is distributed throughout the world and is mostly associated with other viruses as part of the CMD virus complex (Waterhouse and Murant, 1982; Gibbs *et al.*, 1996b; Watson *et al.*, 1998; Vercruysse *et al.*, 2000a). CtRLV acts as a helper

virus for aphid transmission of the other components of the CMD virus complex. For his, the genomic RNA of the other components is encapsidated in coat protein of the helper CtRLV. This mechanism is called transcapsidation (heterologous encapsidation) or genomic masking (Watson *et al.*, 1964, 1998; Elnagar and Murant, 1978; Waterhouse and Murant, 1983).

Three genera are currently recognized in the *Luteoviridae* family: the genus *Luteovirus* with *Barley yellow dwarf virus* as type species; the genus *Polerovirus* with *Potato leaf roll virus* as type species and the genus *Enamovirus* with *Pea enation mosaic virus-1* as type species. *Carrot red leaf virus* has long been a species unassigned within the family (Mayo and D'Arcy, 1999). Recently, however, the complete genome sequence of *Carrot red leaf virus* has been unravelled (Huang *et al.*, 2005, Table 1). The CtRLV genome is 5723 nucleotides in length and contains six large open reading frames resembling the genome structure of viruses within the genus *Polerovirus* (Fig. 1).

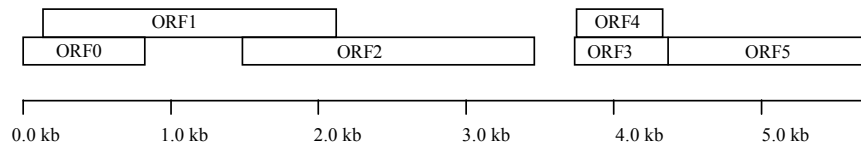


Fig. 1. Genome organization of *Carrot red leaf virus*. Boxes indicate open reading frames. See text for further explanation

Huang *et al.* (2005) have not attempted to define function in any of the ORFs, but analogous products have been assigned functions in some luteoviruses (see Mayo and Miller, 1999 for an overview). ORF0 encodes the putative protein P0; little is known about its function. ORF1 partly overlaps with ORF0 and the product P1 is predicted to be expressed by leaky scanning. The 5' end of ORF2 overlaps with ORF1 and expression of P2 is predicted as an ORF1-ORF2 fusion protein by a minus one frame shift. It has been suggested that P1 and P2 are involved in virus replication, while P2 contains the core domain of RNA-dependent RNA polymerase (RdRp). ORF3 encodes the virion coat protein P3; ORF4 is completely overlapping with ORF3 but in a different reading frame and is suspected to encode the movement protein (P4). ORF5 immediately follows the ORF3 stop codon and seems to be expressed by in-frame translational read-through of the coat protein stop codon. P5 has been suggested to be essential in aphid transmission. Vercruyse (2000) aligned nucleotide and deduced amino acid sequences of CtRLV partial RdRp sequences with corresponding sequences of other luteoviruses. For this gene, the different CtRLV isolates are more similar to *Potato leafroll virus* than to any other luteovirus, and they

are closely related to other members of the *Polerovirus* genus. Since CtRLV is the only luteovirus infecting Umbelliferae, it seems likely that it evolved from a virus that switched host to an umbellifer. Pairwise alignments of the RdRp nucleotide sequences from different CtRLV isolates showed that the CtRLV isolates from Belgium and Scotland are most closely related, while the CtRLV isolates from North America differ considerably from the European isolates (Vercruyse, 2000).

2.2 Carrot mottle virus and Carrot mottle mimic virus

Watson *et al.* (1964) first described *Carrot mottle virus* in Great Britain and CMoV is the type species of the Umbravirus genus. The Umbravirus genus was defined by Murrant *et al.* in 1995, primarily on the basis of two features. First, umbraviruses depend on an interaction with the co-infection luteovirus for their transmission by aphids (Falk *et al.*, 1979; Waterhouse and Murrant, 1983). Second, although umbraviruses have monopartite single-stranded RNA genomes (Falk *et al.*, 1979), infected plants contain two abundant double stranded RNA species of about 4.5 and 1.4 kb (Murrant *et al.*, 1995). Umbraviruses are unusual in that they do not encode their own virion proteins and do not produce their own particles, unlike almost all other viruses (Murrant *et al.*, 1969; Gibbs *et al.*, 1996a; Syller, 2003). They can replicate and move from cell to cell and systematically within plants independent from their helper virus.

In the field, CMoV probably only occurs in mixed infections with CtRLV as a component of the CMD virus complex (Watson *et al.*, 1964). It is transmitted in a persistent manner by the aphid *C. aegopodii*, but only from plants that also contain their luteovirus helper CtRLV. For aphid transmission, the umbravirus genome is probably transcapsidated in the virions of the helper virus. Typical for umbraviruses, CMoV is also mechanically transmissible.

The genome of what was thought to be an Australian isolate of CMoV was sequenced by Gibbs *et al.* (1996a) (Table 1) and was originally named CMoV-strain A. However, Northern hybridisation experiments showed it was distinct from CMoV (Gibbs *et al.*, 1996b) and it was renamed *Carrot mottle mimic umbravirus*. The CMoMV sequence comprises 4253 nucleotides (Fig. 2). Hybridization experiments grouped isolates from Australia, New Zealand and California as CMoMV, while isolates from Great Britain and Morocco were grouped as CMoV. Vercruyse *et al.* (2000a) showed that, at least in Belgium, the umbravirus in parsley with CMD disease is CMoV. Only partial sequences of putative RdRps of CMoV isolates from the UK are available in the database (Accession numbers AY325509 until AY325514).

The genome organization of all sequenced umbraviruses is very similar (see also Figure 2). The genome consists of one linear segment of positive-sense ssRNA and four ORFs can be recognized. ORF1 and ORF2 are translated as a single polypeptide by a mechanism involving a -1 frameshift. The predicted amino acid sequence of the ORF2-encoding part of the protein has similarities with the sequences of RdRp of viruses in the family *Tombusviridae*. A short untranslated region separates ORF2 from ORFs 3 and 4, which overlap each other almost completely in different reading frames. The ORF4 gene product is involved in cell-to-cell movement. The ORF3 gene product functions to protect viral RNA and enables its transport through the phloem (Taliensky and Robinson, 2003).

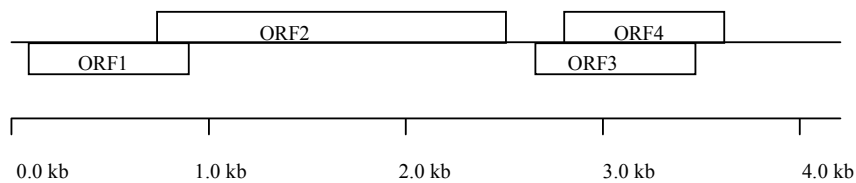


Fig. 2. Genome organization of *Carrot mottle mimic virus*. Boxes indicate open reading frames. See text for further explanation.

2.3 CtRLV associated RNA

Watson *et al.* (1998) showed that virions purified from plants infected with the viruses that cause Californian CMD contained three prominent single-stranded RNAs of approximately 5.6 kb (genomic RNA of CtRLV), 4.2 kb (genomic RNA of CMoMV) and 2.8 kb. Virions also contained an approximately 1.3 kb RNA related to CMoMV RNA. The 2.8 kb RNA did not hybridise with cRNA probes from the Californian CtRLV or CMoMV. Analysis of naturally infected carrot plants showed that CtRLV, CMoMV and the 2.8 kb RNA were always present in CMD-affected plants in California. Transmission experiments showed that the 2.8 kb RNA was never present in plants infected by only CMoMV, but only in plants infected by both CtRLV and CMoMV. The complete nucleotide sequence of this unusual RNA was determined to be 2835 nucleotides (Table 1, Figure 3). Two large open reading frames (ORFs), 1a and 1b, separated by an amber (UAG) stop codon, are present within the sequence and a third ORF (ORF2) is located near the 3' terminus. ORF1a+1b probably represents the RdRP. It is not yet known whether ORF2 encodes for a functional protein. Based on its biological and molecular characteristics, the 2.8 kb RNA was named CtRLV associated RNA.

Carrot Motley Dwarf Virus Complex

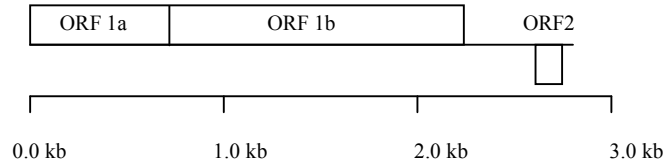


Fig. 3. Genome organization of *Carrot red leaf* associated RNA. Boxes indicate open reading frames. See text for further explanation.

Computer assisted sequence analysis revealed that the 2.8 kb RNA associated with CMD is very similar to the *Beet western yellows virus* ST9-associated RNA (ST9aRNA). The ST9aRNA and CtRLVaRNA are similar in size. Their genomic organizations, nucleotide and deduced amino acid sequences show a high degree of similarity (Watson *et al.*, 1998). Subsequently, CtRLVaRNA has also been reported from parsley (*Petroselinium crispum*) in Belgium (Vercruyssen *et al.*, 2000a) and from carrots in the UK (Morton *et al.*, 2003).

Table 1. Carrot motley dwarf disease-related complete genome sequences available in the databases

Virus	Strain	Accession number	Definition	Reference
<i>Carrot mottle mimic virus</i>	Australian	U57305	DsRNA1, complete genome	Gibbs <i>et al.</i> , 1996a
<i>Carrot red leaf virus</i>	UK-1	AY695933	Complete genome	Huang <i>et al.</i> , 2005
<i>Carrot red leaf associated RNA</i>	Clone a8	AF020616	Putative RdRp, complete cds	Watson <i>et al.</i> , 1998
	Clone a25	AF020617	Putative RdRp, complete cds	

The host range of the CMD disease is restricted to certain species of *Umbelliferae*. The disease occurs worldwide in carrot and has been reported to occur in parsley in Great Britain and Belgium (Watson *et al.*, 1964; Frown and Tomlinson, 1972; Meunier and Verhoyen, 1987; Watson and Falk, 1994; Vercruyssen *et al.*, 2000a). CMD causes reddening and yellowing of carrot leaves with varying amounts of mottling and severe stunting. Yield losses in carrot of 50-60% due to the CMD disease have been reported, but there is considerable year-to-year variation (Watson and Serjeant, 1964; Krass and Schlegel, 1974). Parsley (*Petroselinium crispum* Nym.) plants with reddening and leaf chlorosis associated with severe stunting, resembling those of CMD in carrot, were reported in Great Britain (Frown and Tomlinson, 1972). Similar symptoms have been recorded in Belgian parsley

since the 1980s, and it has since become the most serious threat to parsley production in Belgium (Bleyaert *et al.*, 1987) (Fig 4). The colour change of the parsley foliage, accompanied by stunting, quickly renders the crop unmarketable.



Fig. 4. Parsley field in Belgium seriously infected with the carrot motley dwarf virus complex. Note : reddening and chlorosis of the leaves.

4. MOLECULAR DIAGNOSIS OF THE CARROT MOTLEY DWARF VIRUS COMPLEX

Polyclonal antibodies have been used to detect CtRLV virion protein in chervil (*Anthriscus cerefolium* L.) and carrot (Waterhouse and Murant, 1981; Watson and Falk, 1994). Cross-reaction was found when chervil, artificially infected with the CMD-like disease of Belgian parsley, was tested using these antibodies in DAS-ELISA (Vercruyse *et al.*, 1999a). However, the method was not successful when applied to infected parsley plants, due to inhibiting components in the parsley extracts.

CMoV and CMoMV have been distinguished using cDNA probes (Gibbs *et al.*, 1996b), and the three viral agents found in plants with CMD disease in California have been detected using cRNA and cDNA probes (Watson *et al.*, 1998; Vercruyse *et al.*, 1999a). However, no hybridisation signal was observed when a cDNA generated from the Californian CtRLV was used in Northern hybridisations of nucleic acid from parsley with the Belgian CMD-like disease (Vercruyse *et al.*, 1999a).

Table 2. Primer sequences and their positions relative to the genomic sequences of related viruses

Primer	Target	Sequence ^a	Sense	Position in genome of related viral agent
CtRLV-1	CtRLV	GAGGTGAGAAATCGCYTGAC	+	2753-2772 (CTRLV)
CtRLV-2	CtRLV	MGGCGCCACARTGATAGG	-	2946-2963 (CTRLV)
CtRLVaRNA-1	CtRLVaRNA	TCTAGTTTCTCTCAAGTTCCA	+	1984-2004 (CtRLVaRNA-a8)
CtRLVaRNA-2	CtRLVaRNA	ACCCTCCCCTGTAGTTC	-	2704-2720 (CtRLVaRNA-a8)
CtRLVaRNA-3	CtRLVaRNA	CCTCACCTRCCAATTATGG	-	2451-2469 (CtRLVaRNA-a8)
umbra-NNS	CMoV	TGGWGTICACAACAACACTC	+	1183-1200 (CMoMV)
umbra-FDQH	CMoV	GAIACATGCTGRTCAA	-	1712 - 1728 (CMoMV)
umbra-IBS	CMoV	AAGGCTTTGTACAACATTGG	-	1568-1587 (CMoMV)

^a I = inosine, Y = [C,T], M = [A,C], R = [A, G], W = [A, T]

Vercruyse *et al.* (2000a) developed a reverse-transcription PCR method, beginning with a single cDNA synthesis reaction primed with random hexamer primers, for detecting and identifying the different viral components associated with the CMD disease. A pair of redundant primers umbra-NNS and umbra-FDQH (Table 2) was designed to hybridise to the RdRp gene sequence of all umbraviruses. cDNA fragments of about 550 bp long were amplified when total nucleic acid from chervil with the Belgian CMD were analysed in reactions primed with these primers. Similar cDNA products were generated from extracts from *Nicotiana benthamiana* inoculated mechanically with the Scottish CMoV. No cDNA of the expected size however, was amplified from samples of Belgian parsley when these primers were used, although cDNAs were produced from samples of chervil inoculated with CMD from parsley via aphid transmission. To overcome this problem, an additional internal negative-sense redundant primer umbra-IBS (Table 2) was designed. When umbra-NNS was used in combination with umbra-IBS, cDNA of the expected size (408 base pairs) was obtained from all plants infected with either the Belgian or Scottish isolates of CMoV. The primer sequences umbra-NNS and umbra-IBS not only match sequences in the genome of CMoV, but they also match sequences in the umbravirus genomes of CMoMV, *Groundnut rosette virus* and *Pea enation mosaic virus*.

A pair of redundant CTRLV primers CTRLV-1 and CTRLV-2 (Table 2) was designed based on a partial sequence from a canadian isolate of CTRLV and sequences from five *Potato leafroll virus* isolates. The primers were designed to target conserved regions in the RdRp genes of these viruses. RT-PCR with these redundant primers on nucleic acid preparations from parsley and chervil with CMD disease produced cDNAs of the expected size, i.e. about 211 bp. Products of identical size were produced when RNA from purified PLRV virions was used. Vercruyse *et al.* (2000a) were unable to amplify fragments of CTRLV using the degenerate luteovirus group-specific primers Lu1 and Lu4, that target the coat protein gene (Robertson *et al.*, 1991).

A pair of specific primers CTRLVaRNA-1 and CTRLVaRNA-2 (Table 2) was designed using an alignment of the sequences of the Californian isolates of CTRLVaRNA a8 and a25. The first of these primers targeted a conserved region in the RdRp gene (ORF 1b), whereas the second targeted a conserved region in ORF2 of this virus. An additional redundant negative-sense primer CTRLVaRNA-3 (Table 2) was also designed to target a conserved region in the CTRLVaRNA RdRp gene. Reactions primed with the primers CTRLVaRNA-1 and CTRLVaRNA-2 produced a cDNA fragment that was 781 bp long, which was somewhat longer than the expected size of 737 bp. This cDNA was generated from reactions using nucleic acid extracts from both parsley and chervil with CMD disease. The 781 bp fragment of CTRLVaRNA was only produced when cDNA synthesis

was primed with CTRLVaRNA-2; it was not amplified from cDNA made using random hexamers. Substitution of CTRLVaRNA-2 for CTRLVaRNA-3 in the PCR reactions yielded a product of 483 bp after cDNA synthesis with either random hexamer primers or CTRLVaRNA-2. Primers CTRLVaRNA-1 and CTRLVaRNA-2 were also successfully used by Morton *et al.* (2003) for standard PCR reactions on total RNA extracts of carrot leaves infected with the CMD complex.

All three viruses associated with CMD disease were detected by RT-PCR in individual viruliferous *C. aegopodii*. cDNA synthesis was primed with random hexamer primers; CMoV-derived cDNAs were detected using the primers umbra-NNS and umbra-IBS; CTRLV-derived cDNAs were detected using the primers CTRLV-1 and CTRLV-2 and CTRLVaRNA-derived cDNAs were detected using the primers CTRLVaRNA-1 and CTRLVaRNA-3. When working with very small samples like aphids, an internal control giving a band in the RT-PCR reaction irrespective of the presence of virus is desirable. Vercruyssen *et al.* (2000) showed that actin primers (Canning *et al.*, 1996) can successfully be used to amplify an actin cDNA fragment from *C. aegopodii*. These primers can be combined with for instance CTRLV primers in a duplex reaction.

5. EPIDEMIOLOGY AND CONTROL OF THE CMD DISEASE

5.1 The willow-carrot aphid, *Cavariella aegopodii*, vector of the disease

Since the CMD virus complex is neither transmissible mechanically, nor through seed, its epidemiology is closely linked to the life cycle of its aphid vector, *C. aegopodii*. *C. aegopodii* is a widespread pest of carrot, parsley and other umbelliferous plants (Gratwick, 1992). Direct damage by the aphid is seldom as serious as that caused by transmission of the CMD virus complex. The CMD virus complex is transmitted by *C. aegopodii* in a persistent (circulative), nonpropagative way (Stubbs, 1948; Elnagar and Murrant, 1978). Viruliferous aphids retain the virus complex through the moult, but it is not transmitted to their progeny.

The aphid overwinters in the egg stage on its primary host, the willow tree (*Salix* sp.), which is immune to CMD (Watson *et al.*, 1964). The eggs are laid round the bud axils of willows and hatch in February or March in Great Britain (Dunn, 1965; Gratwick, 1992). Colonies on willows are formed in May and are obligatory non-viruliferous. Winged aphids that appear from these colonies migrate over a period of several weeks to secondary hosts such as carrot and parsley. On these secondary hosts, populations of alienicolae build up to high numbers, and during summer,

other winged generations develop that disperse further to other secondary hosts and back to willows. Many umbellifers die down completely before the winter, but those, which do not can support hibernating forms of the aphid capable of producing colonies in the following spring. Winged forms may arise from these colonies in advance of those developing on willows. Such overwintering is of particular importance for transmission of CMD disease, because any winged progeny of the hibernating aphids is likely to be carrying the virus complex when they arrive to new crops. This was clearly demonstrated by Dunn (1965) who observed that CMD in Great Britain was most troublesome when *C. aegopodii* apterae (wingless forms) survived on overwintering carrot crops.

The timing and size of vector migration flights is of considerable epidemiological significance. In their study on the effect of CMD on the yield of carrots in Great Britain, Watson and Serjeant (1964) found a linear relation with the log mean weekly numbers of winged *C. aegopodii* caught on sticky traps during five consecutive years. In Washington State (USA), the initial appearance of CMD in commercial carrot fields was correlated with May and June flights of *C. aegopodii* (Howell and Mink, 1977b). Van Dijk and Bos (1985) reported that the onset of the CMD disease was 2 to 4 weeks after the start of the May migrations of *C. aegopodii* in the Netherlands.

Since the primary host of *C. aegopodii*, the willow is immune to CMD, first generation aphids on the willows are non-viruliferous. These aphids can acquire the CMD virus complex from four possible primary inoculum sources: 1) umbelliferous weeds; 2) overlapping growing seasons of umbelliferous crops; 3) volunteer crops and 4) crop rotation with other umbelliferous crops (Howell and Mink, 1977a).

In Great Britain, the CMD virus complex is known to occur naturally in the umbelliferous weeds *Anthriscus sylvestris* L. (cow parsley), *Heracleum sphondylium* L. (cow parsnip, hogweed) and *Daucus carota* L. (wild carrot) (Watson *et al.*, 1964; Waterhouse and Murant, 1982). When umbelliferous weeds in Washington State and California were surveyed for the presence of CMD however, natural weed hosts for the virus complex were not found (Howell and Mink, 1977a, Watson and Falk, 1994). Hence, the significance of weed hosts as sources for CMD spread can differ considerably between different geographical regions.

In Washington State, carrots raised for root processing together with volunteer carrots and carrots grown for seed appear to form a continuous yearly cycle of hosts which perpetuated the CMD virus complex (Howell and Mink, 1977a). A close association between the incidence of CMD in spring carrots and overwintered carrot fields was also observed in California (Watson and Falk, 1994).

5.2 Control of the carrot motley dwarf disease in carrots

Control of CMD in carrots mainly depends on cultural methods aimed at reducing sources of CMD and its aphid vector, and are also aimed at breaking the year-round cycle of disease. In carrot-growing regions, willows are too numerous and too large for any practical scheme aiming at reducing this source of *C. aegopodii* (Gratwick, 1992). Although avoidance or removal of umbelliferous weeds near carrot fields is often recommended (Gratwick, 1992), this is impossible or useless in some geographical regions: umbelliferous weeds such as *A. sylvestris* are ubiquitous in the Netherlands (Van Dijk and Bos, 1985), while in Washington State and California, weeds do not seem to be significant sources for CMD spread (Howell and Mink, 1977a; Watson and Falk, 1994). The number of volunteer carrots and overwintered fields should be limited and the newly planted carrot fields should be grown at a substantial distance from the overwintered fields (Dunn, 1965; Howell and Mink, 1977a; Watson and Falk, 1994). In Great Britain, carrots sown in April or May are most at risk, while those sown in June may often escape attack (Dunn, 1965; Gratwick, 1992).

Several insecticides are recommended for chemical control of *C. aegopodii* in carrots. Spray treatments control the aphids more effectively as do granular treatments and the more persistent chemicals have given the best results. However, insecticides are ineffective for control of virus transmission to new crops. When migrations have occurred from old crops, up to seven sprays with a persistent aphicide have failed to control the aphids and the only hope in such a situation is to find the source of aphids and deal with them there. Therefore, it is important to apply the spray treatment also to any overwintered umbelliferous crop left for seed production (Gratwick, 1992). Little is known about the possibilities for biological control of *C. aegopodii* and CMD in carrot. Some resistance to CMD, but not immunity, is available in certain carrot cultivars (Watson and Falk, 1994) and aphid repellent carrot cultivars have been reported (Waterhouse *et al.*, 1986).

5.3 Control of the carrot motley dwarf disease in parsley

For control of the CMD disease in parsley, several cultural and chemical control measures, mainly similar to those for the control of CMD in carrot, are recommended in Great Britain (Gratwick, 1992). However, the use of insecticides is often not successful since parsley is cut several times during the growing season and hence, mainly less persistent chemicals can be used. Covering the crop with a gauze is often recommended for control of the disease, but this is relatively expensive and labour intensive (Bleyaert *et al.*, 1987). Furthermore, due to the frequent cuttings, this method frequently resulted in high levels of infection. No resistant parsley cultivars have been

reported.

Cropping practices that are suggested to control CMD on parsley in Belgium include a later planting date (after the first migration period of *C. aegopodii* at the end of May), removal of volunteer parsley plants, limitation of the number of overwintered fields, and planting of new fields at a considerable distance from the overwintered fields (Vercruyse *et al.*, 1999b). However, avoidance of infection and spread of CMD by later planting is acceptable as a control strategy only when it is compatible with other cropping practices, or when expected yield losses from CMD are greater than the potential gain from early sowing. The role of umbelliferous weeds in the spread of CMD in Belgium is still uncertain. Although it was demonstrated that these weeds serve as reservoirs of vector aphids, their potential role as sources of the CMD virus complex is not clear (Vercruyse *et al.*, 1999b). In addition, removal of umbelliferous weeds is difficult since they are ubiquitous in Belgium, and many wild umbellifers serve as sources of natural enemies of *C. aegopodii*. The use of insecticides for CMD control is only justified in the first part of the growing season (end of May until the end of July), when aphid populations are high and vector migrations are most intensive. Furthermore, insecticides should be selective so that their effects on populations of natural enemies of the aphid vector are minimal (Vercruyse *et al.*, 1999b; 2000b).

6. REFERENCES

- Bleyaert, P., Meunier, S. and Verhoyen, M. 1987. Yellowing of leaves and dwarfism in parsley (*Petroselinum crispum* (Mill.) Nyman ex. A.W. Hill). *Revue de l'Agriculture*, 40(6): 1505-1516.
- Canning, E.S.G., Penrose, M.J., Barker, I. and Coates, D. 1996. Improved detection of barley yellow dwarf virus in single aphids using RT-PCR. *Journal of Virological Methods*, 56: 191-197.
- Dunn, J.A. 1965. Studies on the aphid, *Cavariella aegopodii* Scop. I. On the willow and carrot. *Annals of Applied Biology*, 56: 429-438.
- Elnagar, S. and Murrant, A.F. 1978. Relations of carrot red leaf and carrot mottle virus with their aphid vector *Cavariella aegopodii*. *Annals of Applied Biology*, 89: 237-244.
- Falk, B.W., Morris, T.J. and Duffus, J.E. 1979. Unstable infectivity and sedimentable dsRNA associated with lettuce speckles mottle virus. *Virology*, 96: 239-248.
- Figueira, A.R. 1995. Diseases caused by viruses on Umbelliferae. *Informe Agropecuario Belo Horizonte*, 17: 70-73.
- Frowd, J.A. and Tomlinson, J.A. 1972. The isolation and identification of parsley viruses occurring in Britain. *Annals of Applied Biology*, 72: 177-188.
- Gibbs, M.J., Cooper, J.I. and Waterhouse, P.M. 1996a. The genome organisation and affinities of an Australian isolate of carrot mottle umbravirus. *Virology*, 224: 310-313.

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- Gibbs, M.J., Ziegler, A., Robinson, D.J., Waterhouse, P.M. and Cooper, J.I. 1996b. Carrot mottle mimic virus (CMoMV), a second umbravirus associated with carrot motley dwarf disease recognized by nucleic acid hybridization. Molecular Plant Pathology On-line (URL <http://www.bspp.org.uk/mppl/1996/1111gibbs>).
- Gratwick, M. 1992. Willow-carrot aphid pp. 73-77. In: Crop pests in the UK. Collected edition of MAFF leaflets (ed. M. Gratwick). Chapman and Hall, London.
- Heinze, Z. 1968. Die scheckige Verzweigung der Möhre (carrot motley dwarf) auch in Deutschland. Zeitschrift für Pflanzenkrankheiten, Pflanzenpathologie und Pflanzenschutz 75, 513-517.
- Howell, W.E. and Mink, G.I. 1977a. The role of weed hosts, volunteer carrots, and overlapping growing seasons in the epidemiology of carrot thin leaf and carrot motley dwarf diseases in Central Washington. Plant Disease Reporter, 61: 217-222.
- Huang, L.E., Naylor, M., Pallet, D.W., Reeves, J., Cooper, J.I. and Wang, H. 2005. The complete genome sequence, organization and affinities of carrot red leaf virus. Archives of Virology, 150: 1845-1855.
- Komuro, Y. and Yamashita, I. 1956. Studies on the motley dwarf disease of carrot caused by an insect transmitted virus. Annals of the Phytopathological Society of Japan, 20: 155-160.
- Krass, C.J. and Schlegel, D.E. 1974. "Motley dwarf" virus disease complex of California carrots. Phytopathology, 64: 151-152.
- Marco, S. 1993. Carrot red leaf virus in Israel. Phytoparasitica, 21: 123.
- Mayo, M.A. and D'Arcy, D.J. 1999. Family Luteoviridae: a reclassification of luteoviruses pp. 15-23. In: The *Luteoviridae* (eds. H.G. Smith and H. Barker). CABI Publishing, Wallingford, UK.
- Mayo, M.A. and Miller, W.A. 1999. The structure and expression of luteovirus genomes pp. 23-42. In: The *Luteoviridae* (eds. H.G. Smith and H. Barker) CABI publishing, Wallingford, UK.
- Meunier, S. and Verhoyen, M. 1987. Carrot motley dwarf disease sur carotte et persil en Belgique. Mededelingen Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent, 52: 1019-1025.
- Miller, W.A. 1994. Luteoviruses pp. 792-798. In: Encyclopedia of Virology (eds. R.G. Webster, A. Granoff, A.). Academic Press, London.
- Morton, A., Spence, N.J., Boonham, N. and Barbara, D.J. 2003. Carrot red leaf associated RNA in carrots in the United Kingdom. Plant Pathology, 52: 795.
- Murant, A.F. 1975. Occurrence of mottle and redleaf components of carrot motley dwarf disease in British Columbia. Canadian Plant Disease Survey, 55: 103-105.
- Murant, A.F., Goold, R.A., Roberts, I.M., and Cathro, J. 1969. Carrot mottle - a persistent aphid-borne virus with unusual properties and particles. Journal of General Virology, 4: 329-341.
- Murant, A.F., Robinson, D.J. and Gibbs, M.J. 1995. Genus Umbravirus pp. 785-790. In: Virus taxonomy: the classification and nomenclature of viruses. Sixth Report of the International Committee on Taxonomy of Viruses (eds. F.A. Murphy,

- C.M. Fauquet, D.H.L. Bishop, S.A. Ghabriel, A.W. Jarvis, G.P. Martelli, M.A. Mayo, M.D. Summers). Springer-Verlag, New York.
- Randles, J.W. and Rathjen, J.P. 1995. Luteovirus pp. 379-383. In: Virus taxonomy: the classification and nomenclature of viruses. Sixth Report of the International Committee on Taxonomy of Viruses (eds. F.A. Murphy, C.M. Fauquet, D.H.L. Bishop, S.A. Ghabriel, A.W. Jarvis, G.P. Martelli, M.A. Mayo, M.D. Summers). Springer-Verlag, New York.
- Robertson, N.L., French, R. and Gray, S.M. 1991. Use of group-specific primers and the polymerase chain reaction for the detection and identification of luteoviruses. *Journal of General Virology*, 72: 1473-1477.
- Stubbs, L.L. 1948. A new virus of carrots: Its transmission, host range and control. *Australian Journal of Scientific Research*, B1: 303-332.
- Stubbs, L.L. 1956. Motley dwarf virus disease of carrot in California. *Plant Disease Reporter*, 40: 763-764.
- Syller, J. 2003. Molecular and biological features of umbraviruses, the unusual plant viruses lacking genetic information for a capsid protein. *Physiological and Molecular Plant Pathology*, 63: 35-46.
- Taliansky, M.E. and Robinson, D.J. 2003. Molecular biology of umbraviruses: phantom warriors. *Journal of General Virology*, 84: 1951-1960.
- Van Dijk, P. and Bos, L. 1985. Viral dieback of carrot and other Umbelliferae caused by the Anthriscus strain of parsnip yellow fleck virus, and its distinction from carrot motley dwarf. *Netherlands Journal of Plant Pathology*, 91: 169-187.
- Vercruyssen, P. 2000. Epidemiology, detection and identification of the carrot motley dwarf virus complex on parsley. Ph.D. thesis, Ghent University, Belgium, 119 p.
- Vercruyssen, P., Gibbs, M.J., Tirry, L. and Höfte, M. 1999a. Detection and characterization of the carrot motley dwarf virus complex in parsley. *Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen Universiteit Gent*, 64(4): 25-28.
- Vercruyssen, P., Gibbs, M.J., Tirry, L. and Höfte, M. 2000a. RT-PCR using redundant primers to detect the three viruses associated with carrot motley dwarf disease. *Journal of Virological Methods*, 88: 153-161.
- Vercruyssen, P., Meert, F., Tirry, L. and Höfte, M. 2000b. Evaluation of insecticides for control of *Cavariella aegopodii* and carrot motley dwarf disease in parsley. *Medelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen Universiteit Gent*, 65(1): 9-18.
- Vercruyssen, P., Tirry L. and Höfte, M. 1999b. Seasonal abundance of *Cavariella aegopodii*, vector of a carrot motley dwarf-like disease in parsley. *Medelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen Universiteit Gent*, 64(3a): 251-264.
- Waterhouse, P.M., Garrett, R.G., Johnstone, G.R. and McLean, G.D. 1986. Viruses of plants in Australia. Description of Australian features of viruses. Carrot red leaf luteovirus. URL <http://www.ncbi.nlm.nih.gov/ICTVdb/Aussi/ausidesc.htm>.
- Waterhouse, P.M. and Murant, A.F. 1981. Purification of carrot red leaf virus and

Carrot Motley Dwarf Virus Complex

- evidence from four serological tests for its relationship to luteoviruses. *Annals of Applied Biology*, 97: 191-204.
- Waterhouse, P.M. and Murant, A.F. 1982. Carrot red leaf virus. CMI/AAB descriptions of Plant Viruses no. 249, 4 pp.
- Waterhouse, P.M. and Murant, A.F. 1983. Further evidence on the nature of the dependence of carrot mottle virus on carrot red leaf virus for transmission by aphids. *Annals of Applied Biology*, 103: 455-464.
- Watson, M.A. 1960. Carrot motley dwarf virus. *Plant Pathology*, 9: 133-134.
- Watson, M.A. and Serjeant, E.P. 1964. Effect of motley dwarf virus on yield of carrots and its transmission in the field by *Cavariella aegopodii* Scop. *Annals of Applied Biology*, 53: 77-93.
- Watson, M.A., Serjeant, E.P. and Lennon, E.A. 1964. Carrot motley dwarf and parsnip mottle viruses. *Annals of Applied Biology*, 54: 163-166.
- Watson, M.T. and Falk, B.W. 1994. Ecological and epidemiological factors affecting carrot motley dwarf development in carrots grown in the Salinas Valley of California. *Plant Disease*, 78: 477-481.
- Watson, M.T., Tian, T., Estabrook, E. and Falk, B.W. 1998. A small RNA resembling the beet western yellows luteovirus ST9-associated RNA is a component of the Californian carrot motley dwarf complex. *Phytopathology*, 88: 164-170.



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