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Viral dieback of carrot and other Umbelliferae caused by the Anthriscus strain of parsnip yellow fleck virus, and its distinction from carrot motley dwarf

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Abstract

Viral dieback of carrot, chervil, coriander, dill and wild Umbelliferae is described. Disease incidence in carrot crops grown for seed is often high but low in ware carrot. There is no secondary spread in carrot crops.

The causal virus was identified as the *Anthriscus* strain of parsnip yellow fleck virus (PYFV) transmitted by *Cavariella aegopodii* from cow parsley *(Anthriscus sylvestris). Nicotiana benthamiana* was practically indespensable for isolation of PYFV by sap transmission from plants with viral dieback.

No immunity was found in 12 carrot cultivars or in wild carrot. Disease control with a systemic insecticide had limited effect.

Carrot red leaf virus and carrot mottle virus were commonly found in carrot, but they did not cause dieback symptoms. Cucumber mosaic virus, parsnip mosaic virus and a virus resembling that of carrot yellow leaf were occasionally isolated from carrot. Symptoms due to mycoplasma were also observed.

Additional keywords: Anthriscus yellows virus, carrot mottle virus, carrot red leaf virus, carrot yellow leaf virus, *Cavariella aegopodii,* chervil, coriander, cow parsley, cucumber mosaic virus, dill, helper virus, hogweed, mycoplasma (aster yellows), *Nicotiana benthamiana,* parsnip mosaic virus, virus ecology, wild plants.

Introduction

An early-season dieback of seed crops of carrot *(Daucus carota* ssp. *sativus)* (Fig. 1) was originally reported in the Netherlands in the 1950s as 'het zwart' (blackening) or 'voorjaarsziekte' (spring disease) (Anonymous, 1950, 1955). Diseased plants develop necrosis in axillary shoots followed by death, while tap-roots appear normal. The disease recurs annually and has occasionally led to severe losses in seed production (Anonymous, 1955) and impeded breeding programs (Van Hoof, 1972). Similar symptoms in ware crops have only been reported once (Post-Bakker and Van Hoof, 1959).

[1] Work in partial fulfillment of requirements for master's training at Agricultural University, Wageningen

Since 1949, efforts to isolate the causal agent or demonstrate that the disease was part of the syndrome of carrot motley dwarf (Stubbs, 1948) were unsuccessful.

This publication describes the disease in carrot and other Umbelliferae and distinguishes it from carrot motley dwarf disease, now known to be caused by carrot red leaf virus (CRLV) (Waterhouse and Murant, 1982) and carrot mottle virus (CMotV) (Murant, 1974b). It further reports on the causal virus, the *Anthriscus* strain of parsnip yellow fleck virus (PYFV) (Murant and Goold, 1968), on its ecology and on possible means of control. The disease is now named viral dieback.

Materials and methods

Plant samples. Diseased carrot seed plants were obtained from seed growers or plant breeders, or were sampled at the Institute for Horticultural Plant Breeding (IVT), Wageningen. Samples of carrot plants grown for ware and of other cultivated Umbelliferae were obtained from farmers' fields or private gardens. Wild Umbelliferae were collected along roadsides or were raised from botanical seeds and grown in the open at IPO.

When tested by the Plant Protection Service, Wageningen, samples with dieback symptoms incidentally contained *Alternaria alternata, Botrytis cinerea* or *Fusarium sporotrichoides* unable to cause dieback (H.A. van Kesteren, pers. comm. 1981, 1982 and 1983).

Virus transmission and test plants. Virus transmission from field samples was by *Cavariella aegopodii* to chervil and/or by sap inoculation to *Ammi majus, Chenopodium amaranticolor, C. quinoa, Gomphrena globosa, Nicotiana benthamiana* and *N. clevelandii.*

C. aegopodii was cultured on carrot and chervil plants or, to prevent contamination with CRLV/CMotV, on fennel and parsnip plants. Regular tests proved the aphids to be virus free. Cultures were kept at 20 °C and 16 h/day illumination. Acquisition and inoculation feedings with at least 15 aphids per test were for 1 day each. Aphids were transferred with a small pointed brush, or large numbers of aphids were allowed to move from a wilting infested leaf onto test plants. After inoculation feeding, aphids were killed with pirimicarb (1%) or, when toxic residues had to be avoided, nicotine (1%) with Citowett (0.5%).

For mechanical inoculation plant material was ground (1 g/5 ml) in 0.03 M potassium phosphate, pH 7.7, with 0.1% cysteine hydrochloride. Carborundum 500 mesh was dusted lightly onto the leaves or added to the inoculum (2% w/v). Test plants were grown in an aphid-proof glasshouse with a day and night temperature of 18 °C and additional illumination of 34 000 lux from 400 W SON-T lamps during winter. Plants were usually inoculated at the 5-leaves stage.

Virus isolates and identification. PYFV-*Anthriscus* strain, isolate Dc15 was from a carrot seed plant with typical viral dieback (Fig. 2). Type isolate A421 of PYFV-*Anthriscus* strain and antisera to A421 and the type isolate P121 of PYFV-parsnip strain were provided by Dr A.F. Murant, Invergowrie, Scotland. For serology Dc15 was propagated in *N. benthamiana* and systemically infected leaves were harvested 2 weeks after inoculation. Gel-diffusion tests were with sap clarified by centrifugation at 6 000

rpm for 10 min, or purified by the chloroform-butanol method of Murant and Goold (1968).

Anthriscus yellows virus (AYV) was isolated from cow parsley by *C. aegopodii.* As regards CRLV/CMotV complex, three isolates from carrot with prominent symptoms were used.

As far as identification criteria have not been mentioned under results, these were based on data from the relevant literature on host range, symptoms, aphid transmission and electron microscopy of the viruses concerned.

For electron microscopy preparations were made by triturating a small piece of infected leaf in a few drops of 2% PTA, pH 6.5.

Results

Viruses in carrot and associated symptoms. During 1981-1984 71 seed plants and 73 ware plants of carrot with a wide range of symptoms were tested for virus infection. We obtained 57 isolates which were identified as the *Anthriscus* strain of PYFV (see the section concerned) and 36 isolates of CRLV and/or CMotV.

Incidentally cucumber mosaic virus, parsnip mosaic virus (Murant, 1972), an isolate resembling carrot yellow leaf virus (Yamashita et al., 1976), and unidentified viruses were isolated. Tests with 29 out of 71 of the plants showing mottle or yellowing suggestive of virus infection were negative. From plants with leaf reddening, excessive sprouting and premature bolting, highly characteristic of mycoplasma infection, no virus could be isolated. Also no virus could be detected in 12 mottled carrot seedlings raised in the glasshouse. Large numbers of *C. aegopodii* colonizing a seed plant with viral dieback, when allowed to shift to carrot seedlings, did not transmit PYFV.

Plants containing PYFV often showed necrosis and yellowing in sprouts and umbels (seed plants) or apical leaves (ware plants). However, mottle, yellow mosaic and yellow stem striping at times accompanied by reddening sometimes were the most prominent symptoms, and necrosis did not always occur (Figs 2, 3 and 4). Though some of the plants were additionally infected by CRLV/CMotV, plants with edge reddening or intense overall reddening of young leaves (Fig. 3, left middle) usually were not so. Thus infection by PYFV concurred with symptoms ranging from mild mottle and sometimes reddening to rapid dieback and plant death (Figs 1 and 3). Tap roots appeared normal. However, fibrous lateral roots and the tip of tap roots occasionally showed dieback.

Symptoms caused by natural infections with the motley dwarf viruses were studied in mature ware carrots and seed plants. Plants infected by CRLV and CMotV or by CRLV alone had sturdy lower leaves with interveinal yellowing and edge reddening (Fig. 5, middle) and some showed edge yellowing only. Roots were conical instead of cylindrical and greatly reduced in size (Fig. 6). Symptoms were most severe in the hybrid 'Mokum'. Plants singly infected by CMotV had mildly mottled leaves and their roots were normal in shape and size.
shape and size.

A seed plant infected by cucumber mosaic virus (Fig. 5, right) and a ware plant infected by parsnip mosaic virus showed yellow mosaic. The virus resembling carrot yellow leaf virus, was tentatively identified by the symptoms of its source plant, its closterovirus particles, its poor sap transmissibility which was from carrot to *N. benthamiana,* and its host range different from that of beet yellows virus. The virus could not be reintroduced into carrot by mechanical inoculation.

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Fig. 1. Viral dieback in a carrot seed crop (Photograph H.A. Roggeband, CEBECO).

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Fig. 2. Flowering plant of carrot with characteristic symptoms of viral dieback. Note yellow stem striping and yellowing and browning of axillary shoot, while older leaf remains green.

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Fig. 3. Range of symptoms of viral dieback in branches of flowering carrot plants. From left to right: healthy; overall reddening without necrosis; dieback in main umbel and in axillary sprout of normal leaf; chlorosis in stem of main umbel and of sprout in axil of normal leaf.
Fig. 4. Carrot grown for ware with two plants affected by viral dieback. Note in plant at left the yellowing and epinasty, and in plant at right yellowing and necrosis in youngest leaves, yellowing and edge-reddening of older leaves and absence of symptoms in oldest leaves.
Fig. 5. Leaves of carrot seed plants being healthy (left), naturally infected by CRLV/CMotV (middle: interveinal yellowing and red leaf edges) or by CMV (right: yellow mosaic).

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▼ 5

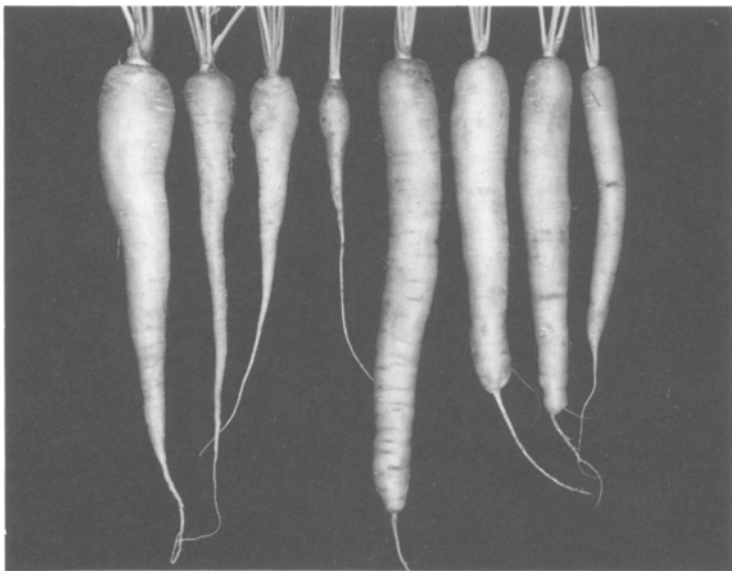

Fig. 6. Tap-roots of carrot plants cv. Mokum numbered 1 (extreme left) to 8 (extreme right). Plants were sampled from a mature ware crop for red-leaf symptoms (1 to 4) or normal appearance (5 to 8). Tests demonstrated CRLV and CMotV in plants 1 and 2, CRLV only in plants 3 and 4, and no virus in plants 5 to 8. Note the smaller average size and conical instead of cylindrical shape of roots 1 to 4 due to CRLV.

Viruses in chervil, coriander, dill and wild Umbelliferae with viral dieback. Viral dieback was not only observed in carrot crops but also in a wide range of flowering wild or cultivated Umbelliferae (Table 1). Plants of *Anethum graveolens* (dill), *Anthriscus cerefolium* (chervil), *Chaerophyllum temulum, Coriandrum sativum* (coriander), *Daucus carota* (wild carrot), *Oenanthe aquatica* and *Torilis japonica* (Fig. 7) with dieback and of *Aethusa cynapium* recovering from necrotic shock were tested by inoculation with sap to *Nicotiana benthamiana.* This resulted in the consistent isolation of the *Anthriscus* strain of PYFV. *C. temulum* was also tested with *C. aegopodii.* Despite its overall reddening and leafrolling in addition to dieback symptoms, suggestive of additional infection by AYV or CRLV, the aphids did not transmit AYV, CRLV or PYFV.

Identification of the Anthriscus strain of parsnip yellow fleck virus (PYFV). When testing carrot plants with dieback symptoms or symptomless plants of *Anthriscus sylvestris* (cow parsley) for virus infection, virus isolates resembling the *Anthriscus* strain of PYFV were obtained. Twenty-five isolates including Dc15 were tested on a number of selected hosts. All of them but two from cow parsley caused reactions similar to those described for the *Anthriscus* strain of PYFV by Murant and Goold (1968), including their lack of systemic reaction in *N. clevelandii,* except for the apical necrosis we observed in *Anethum graveolens* (dill). The two deviating isolates from cow parsley induced faint chlorotic spots in systemically infected leaves of *N. clevelandii* not resembling those of the parsnip strain of the virus (Murant and Goold, 1968).

Fig. 7. Viral dieback in group of plants of *Torilis japonica*.

Further natural umbelliferous hosts are listed in Table 1. Symptoms are described below (see next section).

N. benthamiana appeared to be a new and important test host. Chlorotic local lesions were visible 5 days after inoculation and usually developed a necrotic center, and after a further 4 days the topmost leaves showed vein clearing. With most isolates a progressive necrosis killed the plants within a month (Fig. 8). With some isolates mosaic without necrosis was followed by plant recovery. Other isolates caused rapid necrosis without chlorosis. A421 was less virulent in *Ammi majus* and *N. benthamiana* than most Dutch isolates from carrot or cow parsley.

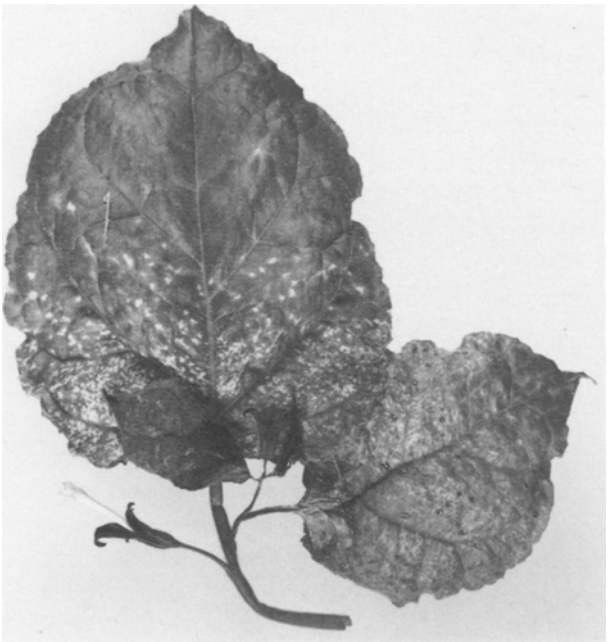

Fig. 8. Systemic veinal necrosis in *Nicotiana benthamiana,* 20 days after inoculation with isolate Dc15 of PYFV-*Anthriscus* strain.

Partially purified Dc15 reacted with antiserum of A421, but not with that of P121 proving it was the *Anthriscus* strain of PYFV. Dc15 did not react in crude sap. Electron microscopy of purified virus showed isometric virus particles, but in crude sap particles were only detected in occasional tubules.

Dc15 could not be transmitted by *C. aegopodii* unless aphids were first fed on chervil plants with *Anthriscus* yellows virus (AYV). This result further supported the identification of Dc15 as PYFV (Murant and Goold, 1968). Besides being helper for the aphid transmission of PYFV, AYV was recognized by its not infecting carrot and causing severe leaf rolling, stunting, reddening and yellowing in chervil and coriander (Fig. 9) as described by Murant and Goold (1968).

Experimental reproduction of viral dieback with PYFV-Anthriscus strain. Carrot cultivars and selections were challenged with the *Anthriscus* strain of PYFV by inoculation with sap or with *C. aegopodii* to fulfill Koch's postulates, and to study the reaction of various cultivars. Aphid transmission was from leaves of cow parsley plants previously found to be a good virus source for the aphid (see Some ecological observations).

After sap inoculation 11 out of 35 flowering plants of the 'Flakkese' selection Flacoro became infected. Symptoms started after 9-30 days. Seven plants inoculated with Dc15 developed yellow or necrotic streaks in the main stem beneath the umbel together with yellowing of young leaves and yellowing or dieback of the axillary shoots. Two months after inoculation the entire plants were yellow or brown. In

Fig. 9. Yellowing, reddening and severe leafrolling in coriander (left) and chervil (right), 34 days and 12 days after inoculation, respectively, with AYV.

Fig. 10. Difference in symptoms between viral dieback (right) and motley dwarf (left) in branches of flowering carrot cv. Mokum, 28 days after aphid inoculation with PYFV-*Anthriscus* strain from cow parsley (right) and CRLV/CMotV from carrot (left).

Table 1. Observations on the natural occurrence of viral dieback in wild and cultivated Umbelliferae

Species with viral dieback	Species without viral dieback
Aethusa cynapium (N)[3]	*Aegopodium podagraria* (N)
Ammi majus (F)[2]	*Angelica archangelica* (C)
A. visnaga (F)	*A. sylvestris* (N)
Anethum graveolens (C)[1]	*Anthriscus sylvestris* (N)
Anthriscus cerefolium (C)	*Apium graveolens* var. *rapaceum* (C)
A. trichosperma (F)	*Carum carvi* (C)
Buleurum rotundifolium (F)	*Cuminum cyminum* (F)
Chaerophyllum temulum (N)	*Foeniculum vulgare* var. *dulce* (C)
Coriandrum sativum (C)	*Heracleum sphondylium* (N)
Daucus carota (N)	*Heteromorpha arborescens* (F)
D. carota ssp. *sativus* (C)	*Pastinaca sativa* (C, N)
Oenanthe aquatica (N)	*Peucedanum palustre* (N)
Orlaya daucoides (F)	*Sium latifolium* (N)
Physocaulis nodosus (F)	*Trachymene pilosa* (F)
Pimpinella affinis (F)	*Trinia ucrainica* (F)
P. anisum (F)	
P. peregrina (F)	
Scandix pecten-veneris (F)	
Tordylium hasselquistiae (F)	
Torilis japonica (N)	
T. leptophylla (F)	
Trachyspermum ammi (F)	

[1] (C) : cultivated species.
[2] (F) : foreign species grown from seed of botanical gardens.
[3] (N) : species in natural populations.

another plant, inoculated with the same isolate, yellowing and leaf reddening with severe necrosis resulted. Infection of 2 other plants by an isolate from a severely necrotic seed plant without yellowing led to identical symptoms and plant death within 30 days. Reintroduction of an isolate from a non-necrotic yellow seed plant caused a similar yellowing without necrosis. Tap-roots of all infected plants remained symptomless except for some root rot caused by carrot fly *(Psila rosea)* which also occurred in control plants. The virus was readily recovered from all 11 infected plants by sap inoculation to *N. benthamiana.* The plants did not contain CRLV/CMotV.

Viral dieback of seed plants also resulted after aphid inoculation of plants of 'Amsterdamse Bak', selection Amsterdamse Vroege Halflange Stomppuntige, the 'Flakkese' selection Flacoro, and 'Mokum' (Fig. 10, right). Symptoms started after 11-30 days. PYFV was readily recovered.

The following cultivars were mechanically inoculated at the seedling stage: Amsterdamse Bak (2 selections), Berlikumer (4 selections), Chantenay Royal, Flakkese (2 selections), Imperator 58 Improved, Karotan, Lange Stompe Winter, Mokum, Nantes (2 selections), Orvita and Wicaro. In comparative tests with Dc15, a second isolate from a necrotic seed plant, and a third isolate from a non-necrotic seed plant, the pro-

portion of plants of the various cultivars and selections becoming infected was low: 34 out of 104, 3 out of 104, and 12 out of 72 inoculated with the isolates, respectively. 'Flakkese' and 'Orvita' were the most susceptible. All cultivars and selections became infected by Dc15.

Symptoms in carrot seedlings started in the youngest leaves 9 days after inoculation and consisted of necrotic vein banding, sometimes extending down the petiole, and malformation. These were followed by yellowing and rolling of neighbouring leaves and epinasty and stiffness of the petioles. Older leaves remained either normal or all leaves wilted after 3 to 4 weeks. Some plants showed mottling, yellowing and some malformation of young leaves with complete cessation of growth without necrosis. Reactions differed between plant replicates irrespective of cultivar, selection or virus isolate. Tap-roots remained normal, but root tips and fibrous lateral roots died, representing some sort of underground dieback. The virus could readily be recovered from necrotic or mottled plants by mechanical inoculation.

Seedlings of mature plants of ware crops of 'Amsterdamse Bak', 'Berlikumer', 'Flakkese' and 'Parijse Broei' also became infected after inoculation with aphids. Symptoms were similar to those above, but infection rates were 100% and the incubation period was mostly 7 days. PYFV was easily recovered from inoculated plants and CMotV was absent.

Apical necrosis and rapid plant death also resulted from sap inoculation of Dc15 to *Ammi majus, Anethum graveolens* (dill), *Anthriscus cerefolium* (chervil), *Coriandrum sativum* (coriander), *Daucus carota* (wild carrot), *Scandix balansae* and *Torilis japonica. Aethusa cynapium* and *Chaerophyllum temulum* reacted with veinal necrosis of the youngest leaves, but plants of *C. temulum* died slowly whereas those of *A. cynapium* recovered. *Pimpinella anisum* (anise) usually showed an apical dieback, but white vein banding occurred in plants of one seed lot.

Failure to incite viral dieback with CRLV/CMotV. No clear symptoms were obtained for 6 weeks of observation in several non-flowering young plants of carrot 'Amsterdamse Bak' (2 selections), 'Berlikumer', 'Flakkese' and 'Karotan', or in 3 flowering plants each of 'Amsterdamse Bak' and 'Flakkese' after inoculation with the CRLV/CMotV complex. Contrary to results of others (Watson et al., 1964; Waterhouse and Murant, 1981; Murant, 1984) no infection was obtained in cow parsley or hogweed. However, young plants of the carrot hybrid 'Mokum', known to be sensitive to motley dwarf in ware crops (P. van Rijbroek, RIVRO, pers. comm., 1983), wild carrot, *Aethusa cynapium, Anthriscus cerefolium* (chervil), *Chaerophyllum temulum* and *Torilis japonica* reacted with leaf reddening, leaf rolling and stunting characteristic of the complex (Waterhouse and Murant, 1982) after 3 weeks. Plants of wild carrot and chervil died when inoculated with CMotV/CRLV using large numbers of aphids. Roots of carrot 'Flakkese' and 'Mokum' were stored at 4 °C for 5 weeks and replanted. Those from infected plants of both cultivars were smaller than those from control plants and the lower part of them decayed. On their tops, leaf sprouting was excessive and the sprouts were stunted and red, while a few plants wilted (Fig. 11).

After inoculation of mature plants of 'Mokum' with CRLV/CMotV (5 non-flowering plants with root diameter of 6-10 cm, and 29 flowering plants), the middle leaves became red and yellow with their largest veins remaining green, but no symptoms appeared in flowering stems or in umbels. Symptoms developed more rapidly

and were more severe in plants grown at 15 °C with additional light (Fig. 10, left) than in plants grown at 25 °C. No necrosis developed during 6 weeks of observation and the two viruses were readily re-isolated.

Some ecological observations. In search of sources of infection for viral dieback 54 plants of cow parsley *(Anthriscus sylvestris)* collected at random in the neighbourhood of Wageningen were tested with *C. aegopodii.* The *Anthriscus* strain of PYFV was isolated from 24 of them. Incidence varied largely with location, and infected plants were usually symptomless.

PYFV also turned out to be prevalent in hogweed *(Heracleum sphondylium).* However all 9 isolates from this species caused systemic ringspotting or mosaic in *N. clevelandii,* characteristic of the parsnip strain of the virus (Murant and Goold, 1968).

Fig. 11. Root decay, plant stunting, leaf discolouration and excessive sprouting (middle and right) and wilting (right) of carrot plants cv. Mokum grown from CRLV/CMotV roots after storage at 4 °C. Left: healthy control.

During 1979-1984 disease incidence in carrot seed crops on the island of Tholen in the south-west (Fig. 1) varied from 10 to 80% (H.A. Roggeband, CEBECO, pers. comm.). The disease appeared to be especially damaging in the seed plants of carrot breeding programs. At IVT in 1971 only 500 out of 7000 plants escaped from disease (Van Hoof, 1972) and in other years infection rates of over 50% were common.

Incidence in carrot ware crops usually is very low, even when seed crops are severely infected. However, 10% infection was observed incidentally. We have no indication of secondary spread in carrot crops.

Viral dieback appeared to be common in many wild Umbelliferae though other species were exempt from the disease despite high natural infection pressure (Table 1). The number of plants infected varied largely with location and species.

We found *C. aegopodii* on cow parsley and all species with viral dieback coincident with the *Anthriscus* strain of PYFV in these plant species. Fig. 12 represents weekly catches of *C. aegopodii* with the suction trap in the south-west of the country where the disease seems most prevalent. Onset of the disease is 2 to 4 weeks after the start of the May migrations of the aphid.

Discussion and conclusions

Of a number of viruses reported in carrot few have been associated with necrosis or plant decline. These were celery mosaic virus (Waterhouse and Murant, 1981),

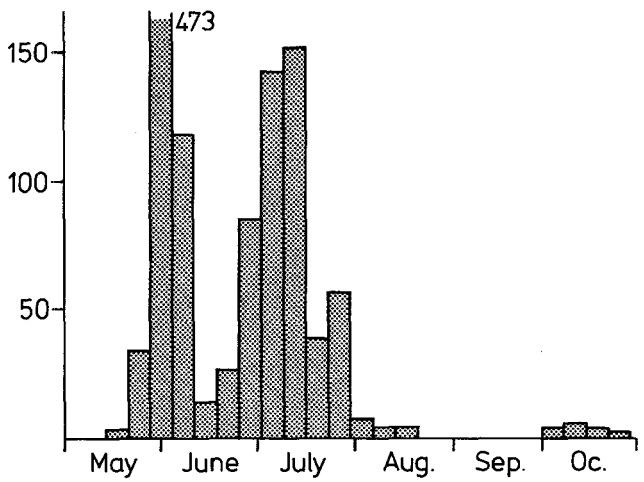

Fig. 12. Weekly catches of *Cavariella aegopodii* with the suction trap at Colijnsplaat in 1979 (5×10^5 m^3 air week^{-1} filtered) (From unpublished data of A.H. van Harten, IPO). Data from Dunn (1965), Dunn and Kirkley (1966) and Dr D. Hille Ris Lambers (pers.comm., 1983) may lead to the following interpretation: The first peak (end of May, early June) shows the migration from willow to carrot and other Umbelliferae. Part of these aphids, after passing cow parsley as a source for PYFV and AYV, transmit PYFV. The second peak (end of June, July) represents further migration to other Umbelliferae although some aphids may continue to spread PYFV from cow parsley. The third peak (October) shows the return to willow.

cucumber mosaic virus (CMV) (Wolf et al., 1969; Van Hoof, 1972) and the CRLV/CMotV complex (Stubbs and Grieve, 1944; Stubbs, 1948, 1956; Heinze, 1968; Howell and Mink, 1979). However, none of these viruses was responsible for such symptoms in our plant samples. In our tests, correlation between PYFV-*Anthriscus* strain and dieback of carrot plants grown for seed or for ware was high, and inoculation experiments leading to artificial reproduction of the syndrome have proved their causal relationship. The disease is now named viral dieback to distinguish it from necrotic diseases caused by fungi or bacteria. In seed plants the disease is characterized by necrosis of axillary shoots and young umbels leading to plant death. Yellowing or reddening of young leaves and yellowing or yellow streaking of young stems may also occur (Figs 2 and 3).

General yellowing and occasional reddening as part of the viral dieback syndrome (Figs 3 and 4) might suggest the involvement or additional involvement of CRLV/CMotV. However, results of virus isolation from carrot cv. Mokum, highly sensitive to CRLV, as well as inoculation experiments with this cultivar at different stages of development, temperatures and light conditions, showed that the reddening of motley dwarf is not prominent in youngest leaves. This disease also is unlikely to be responsible for a necrotic shock reaction in mature plants or for 'leaf scorching' or 'necrotic stem streaking shock symptoms' as reported earlier (Stubbs and Grieve, 1944; Stubbs, 1956). However we could reproduce root rot and plant death (Fig. 11) as reported when tap roots of motley-dwarf-diseased plants are replanted for seed production (Stubbs, 1948; Howell and Mink, 1979). These symptoms essentially differ from those of viral dieback where older leaves remain normal and tap roots sometimes have apical necrosis only.

With CRLV/CMotV yellowing and reddening were not or with difficulty reproduced under glasshouse conditions in tolerant cultivars, whereas wild carrot appeared to be very sensitive. CMotV had little effect on carrot in single infections as also found by Murant et al. (1969), and in mixed infections it does not seem to enhance the symptoms of CRLV (Fig. 6). Heterologous encapsidation of CMotV-RNA in plants with the CRLV/CMotV complex (Waterhouse and Murant, 1983) may explain the incidentally exclusive transmission of CMotV. Our results of virus isolation suggest that CRLV is the major cause of yellowing and reddening of carrot in the Netherlands. The carrot yellow leaf virus, though, may be more prevalent than known so far since it easily escapes detection due to difficult sap transmission.

In Great Britain isolation of PYFV by *Cavariella* spp. has been from cow parsley and hogweed, with the *Anthriscus* strain only occurring in cow parsley and the parsnip strain mainly occurring in hogweed and sometimes in cow parsley (Murant and Goold, 1968; Tomlinson and Carter, 1970; Bem and Murant, 1979). Our results are in line with these findings. Many susceptible Umbelliferae react with dieback and seem to be of little importance, if any, as sources of further spread. In contrast, infection in cow parsley is latent. This tolerance and the perennial nature of the species explain its high level of infection with PYFV-*Anthriscus* strain and helper AYV (Murant and Goold, 1968; our results) so that it may be regarded as the only or main source of infection for viral dieback. Since *C. aegopodii* feeds on cow parsley and carrot, and *C. pastinacae* and *C. theobaldi* do not (Börner, 1952; Dr D. Hille Ris Lambers, pers.

comm., 1983), the first species may be regarded as the exclusive vector of PYFV-*Anthriscus* strain. This species is polyphagous and we have indeed found it on all plant species with viral dieback. When massively migrating from willow during late May and early June it may feed on cow parsley before alighting on carrot and other Umbelliferae (Dunn, 1965; Dunn and Kirkley, 1966; Fig. 12). This migration together with the high incidence of PYFV and AYV in cow parsley explains the epidemiology of viral dieback (Fig. 13). Indeed disease symptoms are noticed 2 to 4 weeks after the onset of the aphid migration. The spread of PYFV from cow parsely may continue at low level during the later part of June and July when *C. aegopodii* migrates for the second time (Fig. 12), as concluded from a slow further increase of the disease during this period.

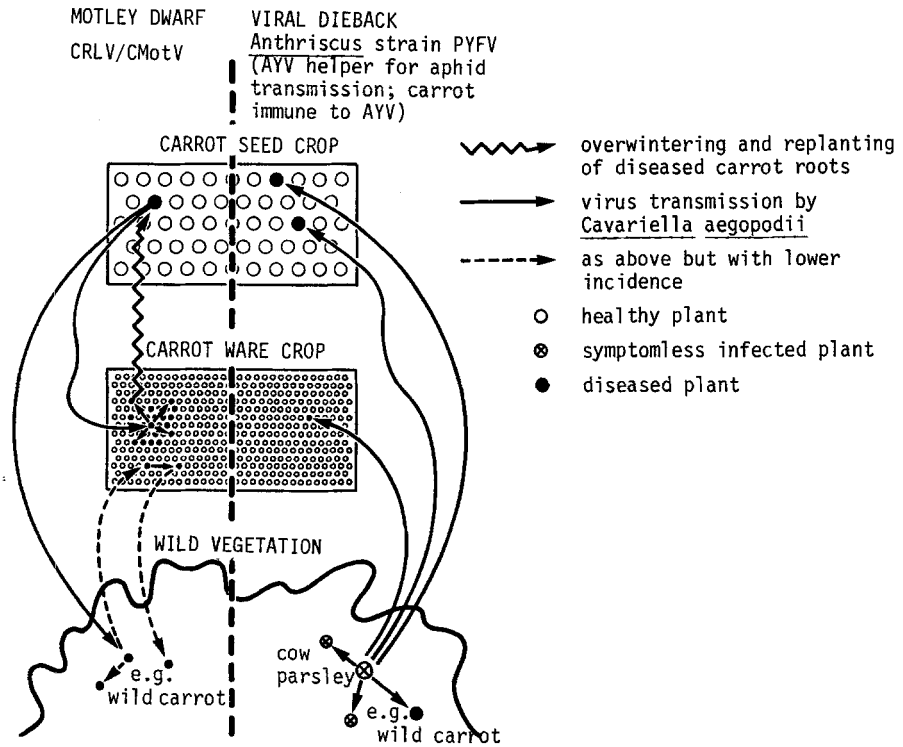

Fig. 13. Comparative epidemiology of viral dieback and motley dwarf of carrot. PYFV-*Anthriscus* strain is spread from cow parsley to carrot and other Umbelliferae by *C. aegopodii* during its migration from willow (Fig. 12). Secondary spread of the virus from carrot is prevented by immunity of carrot to the helper virus AYV. With lack of secondary spread, difference in plant density and possibly in attractiveness to the vector explain why seed crops are affected in much higher proportions than ware crops. In contrast, spread of CRLV/CMotV by *C. aegopodii* is mainly secondarily within the crop. Primary infection may be from wild Umbelliferae and especially from seed plants grown from infected tap roots replanted after overwintering. Extensive cultivation of carrot and particularly the vicinity of carrot crops grown for seed promote the occurrence of CRLV/CMotV but not of PYFV.

Since carrot is insusceptible to AYV (Murant and Goold, 1968; our experiments), carrot infected by PYFV can only act as a source of virus for aphids that previously acquired AYV from other plant species (Waterhouse and Murant, 1981). This explains why we could not isolate the virus with *C. aegopodii* from carrot plants with viral dieback and why there is no further epidemic build up of dieback within carrot crops (Post-Bakker, 1957) after initial infection from outside sources. Hence, removal of infected carrot plants to prevent spread within the crop and increase of inoculum pressure in the wild vegetation is unnecessary.

Disease incidence in carrot ware crops is low, even when seed crops are severely affected. This discrepancy may be atributed to the fact that root crops consist of much more plants per unit area than seed crops. Thus a much smaller proportion of root plants are subject to infection through incoming aphids, while secondary spread is nil. Indeed, the number of *C. aegopodii* is usually much smaller on carrot ware plants than on plants grown for seed (Dr D. Hille Ris Lambers, pers. comm., 1984). Lower attractiveness of the former crop type to the alighting vector might be an additional factor (Fig. 13).

Semi-persistent transmission of the virus by the vector, requiring 2 min to 24 h for inoculation feeding (Murant, 1974a), explains the limited effect of a systemic insecticide (Demeton) in repeated experiments at Alkmaar (Table 2). These results further exclude the involvement of the persistently-transmitted CRLV differing basically in its ecology from PYFV-*Anthriscus* strain (Fig. 13).

As cow parsley is ubiquitous in the Netherlands, avoidance or removal of sources of infection is impossible. However, population densities of the vector differ considerably between parts of the country. Accumulated annual catches in three suction traps from South to North of the country (in Zeeland, the IJsselmeerpolders and Groningen) are in the proportion of 235 : 100 : 6 (data by Ing. A. van Harten and Ms J.D. Prinsen). Thus, the disease may be avoided by cultivation in northern parts of the country.

Breeding of carrot for resistance may not be worthwhile since the disease seems to

Table 2. Effect of treatment with a systemic insecticide on the percentage of carrot seed plants with dieback*.

Treatment	Year			
	1960	1961	1962	1963
Untreated	35	19	0	47
Limited spraying**	15	7	0	27
Frequent spraying***	7	3	0	7

* Data from Van Bakel and De Kraker (1961, 1962, 1963 and 1964). Plants treated with Demeton (1% v/v). Regular tests for fungal cause of dieback were negative. Only necrotic flowering plants were scored as infected but the actual percentage of plants with viral dieback disease was about 1.5 times as high (interpreted from J.M.M. van Bakel, unpublished results 1960 and 1961 and pers.comm., 1984).

** The first spraying in April with some additional spraying when aphids were found on the plants.

*** Spraying with 10-days intervals during April, May and June.

have no economical importance in ware crops. Moreover, immunity could not yet be found in wild or cultivated carrot.

In this study viral dieback of carrot and other Umbelliferae could be distinguished from motley dwarf disease in symptoms, incitant and epidemiology. The delay in properly diagnosing viral dieback in the Netherlands, leading to confusion with motley dwarf, may now be attributed to the fact that isolation of the *Anthriscus*-strain of PYFV from naturally infected plants without the use of *N. benthamiana* is difficult. Control of the disease remains nearly impossible despite our revealing its etiology and epidemiology. The dependance of PYFV on AYV for aphid transmission, and immunity of carrot to AYV are most interesting from an epidemiological point of view. They seem to prevent viral dieback from being economically important in carrot ware crops.

Samenvatting

Virusinsterving van peen en andere schermbloemigen veroorzaakt door de fluitekruidstam van het pastinakegeelvlekvirus en zijn verschil van de voorjaarsziekte

Bij de zaadteelt van peen is in ons land reeds lang een schadelijke, vroeg in het seizoen optredende instervingsziekte bekend als 'voorjaarsziekte' of 'het zwart'. Planten vallen op door necrose van jonge spruiten (insterving). Soms gaat meer dan de helft van het gewas verloren. Voor consumptie geteelde peen wordt echter nauwelijks aangetast. De ziekte is nu ook gevonden bij dille, kervel, koriander en wilde schermbloemigen.

Uit zieke planten en ook vaak uit symptoomloze fluitekruidplanten werd een virus geïsoleerd waarmee de insterving kon worden gereproduceerd. Het werd herkend als de fluitekruid- (of *Anthriscus*-)stam van pastinakegeelvlekvirus (PYFV) op grond van waardplanten, symptomen, serologie en overdracht door *Cavariella aegopodii* met als onmisbare helper het *Anthriscus*-vergelingsvirus (AYV), dat ook in fluitekruid voorkomt. Het gebruik van *Nicotiana benthamiana* als toetsplant maakte isolatie uit planten met virusinsterving mogelijk. Voor de ziekte wordt nu de naam virusinsterving van schermbloemigen voorgesteld.

Peenroodbladigheid veroorzaakt door peenroodbladvirus, dat meestal samengaat met peenvlekkenvirus, bleek ook algemeen voor te komen. Deze twee virussen spelen geen rol bij het veroorzaken van virusinsterving, zoals wel werd aangenomen. Beide ziekten zijn geheel verschillend in symptomatologie en epidemiologie. Incidenteel werden komkommermozaïekvirus, pastinakemozaïekvirus en een virus gelijkend op peengeelbladvirus in aangetroffen. Ook werd eenmaal een aan een mycoplasma toe te schrijven ziekte geconstateerd.

Virusinsterving bleek epidemiologisch te kunnen worden verklaard door de massale jaarlijkse migratie van *C. aegopodii* in het voorjaar, waarbij PYFV van fluitekruid naar peen en andere schermbloemigen wordt verspreid. Door onvatbaarheid van peen voor het helpervirus (AYV) treedt in dit gewas geen secundaire verspreiding op.

In geen van 12 peenrassen en wilde peen werd resistentie aangetroffen. Toepassing van een systemisch insekticide bleek in eerder onderzoek slechts een beperkt effect te hebben. Peenzaadteelt in gebieden met minder bladluizen, zoals het noorden des lands, lijkt aan te bevelen, maar verder lijkt de ziekte niet te bestrijden.

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