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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

Novel viruses detected through previous projects have a national and international distribution in carrots and/or weeds. The incidence of these viruses is variable with season and region.

Carrot yellow leaf virus (CYLV) and Carrot torrado virus (CaTV) have been transmitted by both willow-carrot aphid (*Cavariella aegopodii*) and peach-potato aphid (*Myzus persicae*). Control of peach-potato aphid should be considered within future carrot virus management programmes.

Background

Arising from previous carrot virus studies (FV 382, FV 382a and Adams et al., 2014), several key knowledge gaps were identified in the understanding of carrot virus epidemiology. Closing these gaps will allow the industry to better understand the effects of viral infection in carrot fields and in doing so move from a reactive to a proactive approach to virus management. The fundamental principles of plant virus management are:

- Plant clean seed
- Grow in absence of vectors
- Grow in absence of virus reservoirs
- Isolate from similar crops
- Use resistant, or tolerant, varieties

Although these points were formulated for virus management in seed potato crops, the key principles are transferable to any crop. The first four of these principles have been investigated as part of the work reported here.

The importance of *Parsnip yellow fleck virus* (PYFV) and *Carrot red leaf virus* (CtRLV) as viruses causing economic damage have been recognised in Europe for over 20 years due to the foliar symptoms (CtRLV) and viral die-back of seedlings (PYFV). However, with the recent association of the little studied *Carrot yellow leaf virus* (CYLV) as the causal agent of internal root necrosis, the industry faces a third major viral threat. Additionally there were further novel viruses found during FV 382a namely Carrot torrado virus (CaTV) and Carrot closterovirus-1 (CtCV-1). As these viruses were previously unknown there is limited knowledge about their epidemiology, aetiology, incidence and impact. Consequently there is also limited information that can be given to growers regarding the potential threat from these viruses and how best to manage crops to reduce their incidence. The aim of this project was to start to close the knowledge gaps regarding these recently emerged viruses.

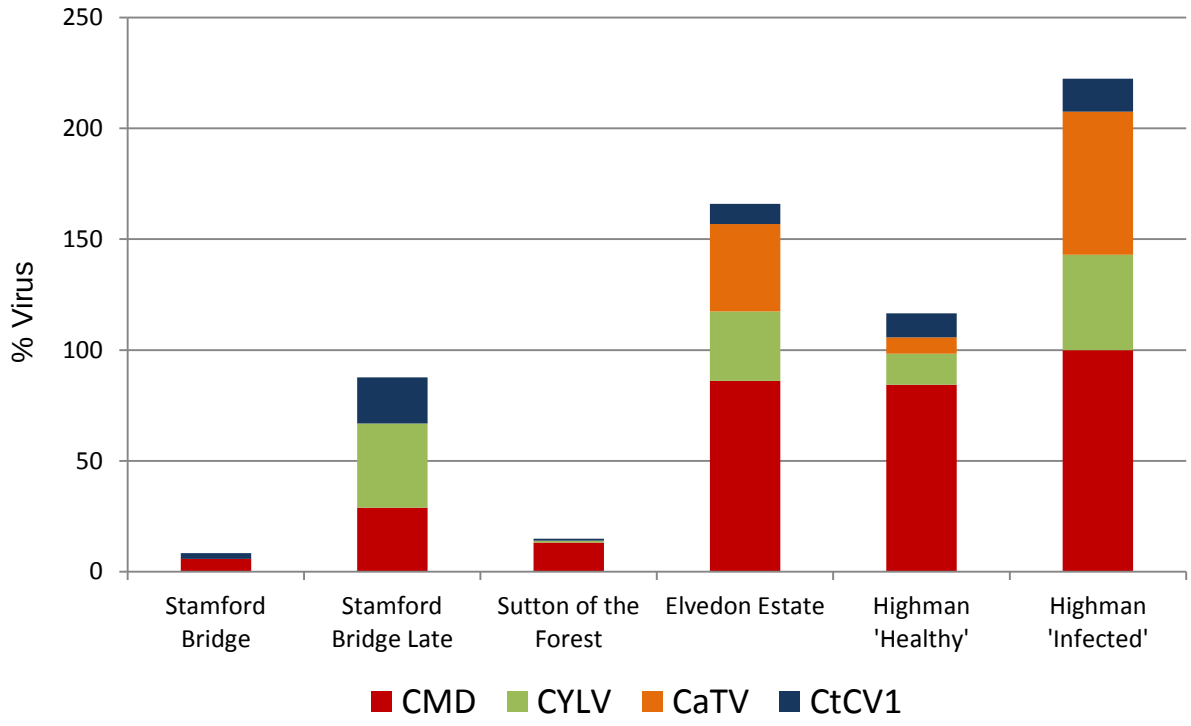
Summary

CYLV was transmitted with low efficiency by the willow-carrot aphid (*Cavariella aegopodii*) the peach-potato aphid (*Myzus persicae*) and the willow-parsnip aphid (*Cavariella theobaldi*), although the virus could not be transmitted early enough within the project to attempt a demonstration of a link to root necrosis through a full carrot growth cycle. Work related to this project has also shown that the virus CaTV is transmitted by aphids, a first demonstration of aphid transmission for a torradovirus. Through this project work there has been a demonstration of *Myzus persicae* as a potential virus vector in carrots as well as the potential for other *Cavariella* species to also play a role in carrot virus epidemics. This will have implications for aphid control strategies as this the peach-potato aphid is known to have has multiple aphicide resistance mechanisms and management of this species presents major challenges for season long control of aphids. Additionally the current resistance status of willow-carrot aphid is unknown. During the course of the project there was evidence of high numbers of aphids in infected crops indicating that current aphid control measures were not wholly effective. The resistance status of willow-carrot aphid and the relative importance of peach-potato aphid as a vector in carrot crops should be investigated in future work.

The novel virus Carrot closterovirus-1 (CtCV-1), as well as Carrot yellow leaf virus (CYLV) and other carrot viruses appear to have a national distribution in both field crops (see Figure A) and alternate host sources. This is a first demonstration that these viruses are present across the UK and in Europe, outside of the previously studied geographic area.

In some fields all carrots sampled were positive for at least one virus and in most cases infected carrots were harbouring multiple virus infections. The presence of carrot viruses in fields at very high incidences gives a high potential for onward transmission of these viruses to infect other carrot crops within the growing season. If these crops were then to be stored under straw in the field this would give the potential for over-wintering of both the virus and the aphid vectors, particularly peach-potato aphid. An overlap between the stored crop and successive crops in nearby fields would, therefore, give a potential risk for these stored crops to form a source of inoculum for young emerging crops.

Figure A. Proportion of viruses in carrot fields in Yorkshire and Norfolk. Data are presented as cumulative % virus in 120 carrots sampled per field.



Carrot torrado virus was notably absent from weed sources but present within carrot crops in both sampling years. There are two possible explanations for this: there is another, as yet unidentified, environmental source of CaTV; or alternatively, CaTV may be a virus which circulates within carrot crops either originating from previous carrot crops or being brought into crops with seed and being spread from within the crop. The potential for viruses to circulate within carrot crops allowing infections to bridge seasons has been previously discussed as a potential transmission route for CtRLV and the CMD complex. From these data there is a distinct possibility that this is also a route by which CaTV is being transmitted. However, the role of seed-borne infections should not be discounted as even a low rate of onward transmission from infected seed could give rise infection sources within carrot fields. Any future work should also include investigations aimed to identify the relative importance of seed as a source of virus infection in carrots and the role played by overwintering crops in carrot virus epidemics.

If we reconsider the fundamental principles of plant virus management in the light of these new data we can now say:

- Plant clean seed: There is a potential that carrot seed may be a source of CaTV
- Grow in absence of vectors: *Myzus persicae* must now be considered as a vector of carrot viruses along with other *Cavariella* species including *C. aegopodii*.
- Grow in absence of virus reservoirs: Both common weed hosts and carrot crops may form a source of virus infections into uninfected crops.
- Isolate from similar crops: The separation of crops in this respect could be isolation from other carrot crops ‘in time’ as well as geographic isolation.
- Use resistant (or tolerant?) varieties: There is still limited data on the susceptibility of a range of varieties. The majority of work reported here has been carried out on cv. Nairobi as this is the variety most commonly grown in the UK.

Although the link between CYLV and necrosis could not be experimentally demonstrated within this project, the virus has been shown to be aphid transmitted. Work related to this project has shown that the virus CaTV is transmitted by aphids, a first demonstration of aphid transmission for a torradovirus. The novel viruses CtCV-1 and CaTV, as well as CYLV appear to have a national distribution in both field crops and alternate host sources, giving potential for transmission into carrot crops as well as other members of the apiaceae, such as field grown herbs. They also appear to be present in carrot fields in Europe and may therefore be of international importance in carrot production. The role of weeds in the epidemiology of these viruses remains to be confirmed, however, they may play an important role as sources of CYLV and CtCV-1. From these data weeds appear to be of limited significance as a source of CaTV infections. The finding of greatest significance for growers is the demonstration of *Myzus persicae* as a potential vector in carrots. This will have implications for aphid control strategies as this aphid species has multiple aphicide resistance mechanisms and will present major challenges for season long control of aphids.

Financial Benefits

At this stage it is difficult to give a clear cost-benefit to growers as the cumulative impact of carrot viruses on the UK carrot industry is still unclear. Virus associated losses to the industry will come from two sources:

a) Necrosis within roots leading to crop rejections as affected roots will be unsuitable for market.

b) Virus infections will lead to a reduced yield due to loss of photosynthetic area in affected foliage.

With a better understanding of the sources of carrot virus epidemics and the key vectors systems can be developed to minimise virus infections and consequently reduce both yield and quality losses.

Action Points

Although the relative importance of each vector aphid species and each virus source is not known, the demonstration of a range of aphid species transmitting viruses to carrots must be considered when formulating aphid/virus management programmes:

- The peach-potato aphid (*Myzus persicae*) appears to be as efficient at transmitting carrot viruses as the willow-carrot aphid (*C. aegopodii*). This species is recognised as having multiple aphicide resistance mechanisms. Additionally other Cavariella species, the parsnip aphid (*C. pastinaceae*) and the willow parsnip aphid (*C. theobaldi*) may also play a role in carrot virus transmission. These aphid species and aphicide resistance management should be considered within aphid monitoring and virus control programmes.
- Due to the limited number of chemical control options available to growers, it will be difficult to maintain a season long virus control programme. Alternate virus management methods should also be employed to give an integrated control strategy. These approaches could include fleece coverage of young crops as a barrier to infection; IPM approaches to increase natural predators; use of aphid monitoring programmes to better target spray application; isolation of crops from virus sources if possible.

Overview of carrot viruses

Virus names are only written in italicised script once they are formally recognised by the International committee on Taxonomy of Viruses (ICTV). Generally speaking plant viruses are named using the following convention:

Common name of initial host – Symptom observed – Virus

This can lead to some confusion if a virus has multiple host species e.g. Cucumber mosaic virus has over 1200 known hosts; or when a symptom is idiosyncratic to a particular variety or is a temporary reaction. Virus nomenclature has been further complicated by the use of novel sequencing techniques, such as those used in FV 382a, where previously unknown viruses are discovered with no direct reference to symptomatic context. In these cases the virus is named after the genus level to which it can be assigned. If the virus is from a novel genus, then it is named after the 'new' genus name.

The information below is designed to give an overview of the viruses referred to in this report.

Parsnip yellow fleck virus **(PYFV)**

PYFV is important as an early season disease where it is associated with seedling death (see Figure B). The virus requires *Anthriscus yellows virus* (AYV) for transmission, and this second virus provides a 'molecular glue' to enable the PYFV to be retained within the aphid foregut. The aphid can be thought of as a 'flying syringe' drawing up the virus and then passing it on through subsequent feeding activity. Transmission is rapid, typically taking less than a few minutes to pass on the virus. The main vector of this virus is considered to be *Cavariella aegopodii*, the willow-carrot aphid.

Figure B. Seedling death in carrots caused *Parsnip yellow fleck virus*

AHDB-Horticulture project FV 228a demonstrated that the source of PYFV infections in carrots are most likely to be associated with cow parsley (*Anthriscus sylvestris*), a common hedgerow weed. As carrots are not a host of AYV, once they are infected with *Parsnip yellow fleck virus* the virus cannot be passed on.

In many seasons, PYFV does not commonly occur. The reasons for this sporadic occurrence are still unknown, but it is possibly due to its complicated epidemiology involving AYV, which limits onward spread in carrot crops. Work conducted at Warwick Crop Centre suggested a close relationship between observed symptom, root weight and the proportion of plants infected with PYFV suggesting that this virus can cause stunting in mature carrot crops (Dez Barbara, pers. comm.).

Carrot Motley Dwarf disease (CMD)

Carrot Motley Dwarf (CMD) is a disease complex comprising of *Carrot red leaf virus* (CtRLV), *Carrot mottle virus* (CMoV) and Carrot red leaf associated viral RNA (CtRLVaRNA). The disease complex can only be transmitted if CtRLV is present as the other two viral components are enclosed within the CtRLV virus particle during aphid acquisition and transmission. However, the individual component pathogens can be found in single infections. The virus is taken up by the aphid and passes through the gut and into the salivary gland where it can be passed on through feeding activity. This process can take several hours. Carrot infections are thought to originate from other carrots rather than weed hosts.

Figure C. Leaf reddening and dwarfing caused by infection with CMD disease complex

CtRLV is associated with leaf reddening (see Figure C) and CMoV with mottling which is a dappled yellowing of the leaf. However, in experimental studies, single infections by either of these viruses resulted in mild symptoms. The two viruses in co-infection have a greater effect on the plant and the result is called carrot motley dwarf disease. The third virus in the complex, CtRLVaRNA, is not known to have any noticeable effect on disease symptoms.

While foliar symptoms may be obvious, there is little data on root symptoms or crop loss due to these viruses. Anecdotally infections with CMD have been linked to root symptoms such as excessive lateral root hairs (bearding) and root cracks and splits (splitting or kippering). Visual identification of this disease complex is not helped by leaf reddening etc. being a general response to stress or physical damage and there are also similar symptoms caused by infections with other pathogens such as phytoplasmas.

Carrot yellow leaf virus (CYLV)

Although this virus has been known to occur in the UK since 1980, very little research was conducted into the virus as it was considered a minor issue. However, FV 382a demonstrated that this virus was strongly associated with carrot internal necrosis (Figure D).

Figure D. Carrot root cross section showing presence of internal necrosis associated with infection from CYLV

The virus is known to be transmitted by a similar mechanism to PYFV, where the virus is sucked into the foregut of the aphid and can be rapidly transmitted into a new host. Unlike PYFV, *Carrot yellow leaf virus* does not require a helper virus and onward transmission in carrot crops will occur. Foliar symptoms are thought to be an upright growth habit and yellowing of foliage (see Figure E). The virus was previously known to be transmitted by *C. aegopodii*, the willow-carrot aphid, *C. pastinaceae*, the parsnip aphid, and *C. theobaldi*, the willow-parsnip aphid. Transmission work carried out during this study has also demonstrated

the ability of *Myzus persicae*, the peach-potato aphid, to transmit the virus. This study has shown the virus to be present in a wide range of apiaceous weed hosts as well as carrot crops, however, the relative importance of each virus source is not yet known.

Figure E. Yellowing of foliage caused by viral infection in carrot. As this carrot contained multiple viruses the symptom cannot be definitively linked to infection with CYLV.

Carrot clostero virus-1 (CtCV-1)

This virus was first described through sequencing findings during FV 382a. Genetically the virus is very similar to CYLV, and is assumed to have a similar biology. Vectors, modes of transmission and field symptoms have not yet been confirmed for this virus. The findings of this study are the first step in showing this virus is widespread in weeds and carrots both in the UK and further afield. Further biological characterisation work is ongoing.

Carrot torrado virus (CaTV)

This virus was also first described through sequencing findings during FV 382a. This virus belongs to a recently discovered genus, the torradoviruses (van der Vlugt, 2015). Most members of this genus are tomato affecting viruses, and CaTV is the first virus in this group to affect the *Apiaceae*. The tomato infecting torradoviruses are known to be whitefly transmitted. The results of this study represent the first demonstration of aphid transmission of a torradovirus, with the virus being shown to be transmissible by both *C. aegopodii*, the willow-carrot aphid, and *M. persicae*, the peach-potato aphid. The virus is not currently thought to cause an observable symptom, but may contribute to yield reduction. As the virus was not

detected from any of the weeds tested in this study it is likely that infected carrots are the source of carrot epidemics. Seeds were shown to be contaminated with the virus, but further work is needed to demonstrate the importance of seed-borne infections.

SCIENCE SECTION

Introduction

Arising from previous carrot virus studies (FV 382, FV 382a and Adams et al., 2014), several key knowledge gaps were identified in the understanding of carrot virus epidemiology. Closing these gaps will allow the industry to better understand the effects of viral infection in carrot fields and in doing so move from a reactive to a proactive approach to virus management.

The fundamental principles of plant virus management are:

- Plant clean seed
- Grow in absence of vectors
- Grow in absence of virus reservoirs
- Isolate from similar crops
- Use resistant (or tolerant?) varieties

Although these points were formulated for virus management in seed potato crops, the key principles are transferable to any crop. Whilst it is not possible in a 'real world' situation to completely eliminate vectors, such as aphids, or alternate host reservoirs; minimising the exposure of the crop to these various factors should give improved control of virus epidemics. Considering the relative importance and influence of these factors in a given cropping system could allow the development of decision support system for carrot growers in managing the impact of virus disease in carrots.

With the limited insecticidal chemistries currently available, the carrot industry is facing an increasing challenge managing the problems caused by virus infection. Of the viruses

currently affecting carrot crops, *Parsnip yellow fleck virus* (PYFV) has been recognised as an early season threat, leading to seedling death, whilst the Carrot Motley Dwarf (CMD) complex has been linked to problems occurring later in the season. This means the limited aphid management options available to growers is already stretched in providing season long prophylactic treatment.

The importance of PYFV and CtRLV as viruses causing economic damage have been recognised in Europe for over 20 years due to the foliar symptoms (CtRLV) and viral die-back of seedlings (PYFV) (Dijk and Bos, 1989) and these viruses have been the primary focus of carrot virus research and diagnostics. However, recent AHDB-Horticulture funded research (FV 382a) demonstrated the little studied *Carrot yellow leaf virus* (CYLV) as the causal agent of internal root necrosis (See Figure 1.) , the industry faces a third major viral threat. Additionally there were further novel viruses found during FV 382a namely Carrot torrado virus (CaTV) and Carrot closterovirus-1 (CtCV-1) (See also Adams et al., 2014). As these viruses were previously unknown there is limited knowledge about their epidemiology, aetiology, incidence and impact. Consequently there is also limited information that can be given to growers regarding the potential threat from these viruses and how best to manage crops to reduce their incidence.

Figure 1. Cross section of carrot root showing internal necrosis

To begin to gather the information required to give growers effective virus management advice, a programme of work was designed to address several of the key knowledge gaps

i) Grow in absence of vectors

Fera has recently completed a project for AHDB-Potatoes (Project R428) which has given experience of working with a 'standardised method' (after Verbeek *et al*, 2010) for comparing the relative efficiency of transmission i.e. where the aim would be to look at not just whether an aphid species can transmit a virus, but to gauge the relative efficiency of that species in transmitting the virus against the most efficient transmitter within the experiment.

Carrot and cow parsley plants were collected from the field following confirmation of virus infection and used as infection source plants to try and demonstrate aphid transmissibility of CYLV. In a co-experiment carried out under a Defra funded PhD study the similar work was carried out for Carrot torrado virus (CaTV).

ii) Grow in absence of virus reservoirs

Alongside a knowledge of which vectors are transmitting the virus, it is essential to understand sources of virus and their influence on epidemiology. For PYFV carrot appears to be a 'dead-end' host however, this is not the case for CtRLV and CYLV. It is known that CYLV can be found in Hogweed (Bem and Murant, 1979), but there has been no large scale surveillance carried out for CYLV to look at the relative importance of different sources of this virus. Using diagnostic tests developed for CYLV and CtCV-1 under FV 382a, and further diagnostics developed under Defra funding (Rozado, submitted for publication)

RNA extracts from weed samples collected under the previous AHDB funded project were tested for CYLV and other carrot viruses.

To give full UK coverage of this work further weed sampling was required. Given the previous focus on apiaceous weeds (mainly Hogweed and Cow Parsley) these were considered to be

the likely alternative hosts. However, whilst these would be expected to be the primary sources of the virus, a broader weed survey should be carried out.

During the course of the project Fera obtained nearly 1500 RNA extracts from apiaceous weeds collected by the late Dez Barbara at Warwick Crop Centre. These samples were included in virus screening for the novel viruses to give a comparative historic dataset covering the viruses traditionally tested for, CtRLV and PYFV, as well as the novel viruses identified during FV 382a.

iii) Isolate from similar crops

During the previous project work was carried out in depth on a limited number of fields in an area known to be virus infected. This raised questions about the geographic distribution of these viruses in carrot crops. To address this, a method was developed to sample fields in a measurable way to compare virus content throughout the season.

Additionally, a diagnostic service was provided to growers and agronomists during the project to test affected carrot plants for the presence of viruses. A limited number of samples were also obtained from non-UK (EU) sources, to confirm whether these viruses were also present in the broader European carrot crop.

iv) Plant clean seed

Carrot viruses are not thought to be seed transmitted, but CtRLV has been found previously during export certification testing. Using the real-time PCR assays for CYLV and CaTV it was proposed to test carrot seeds to ensure that this is not a potential route for virus entry into carrot crops.

The initial aim of this project was to attempt to confirm the causal relationship between CYLV and root necrosis following the association made in FV 382a. Due to the difficulties of working with viruses which could only be transmitted by aphid vectors in a challenging experimental crop such as carrot the initial work on CYLV was limited to allow further work of value to the

grower to be carried out investigating field sources of the virus and confirming that the virus problems observed in Yorkshire in the previous project were not regionally limited. The work described here relating to CaTV has been gathered through a parallel Defra-funded PhD studentship and is presented here as contribution in kind.

Materials and methods

1. Diagnostic testing

Throughout the project samples arising from all aspects of the work were tested by real-time PCR. The details for the general testing by this method as follows:

1.1. Nucleic acid extraction

The extraction of viral RNA was performed using the in-house Fera magnetic bead

method.

1.2. Real-time PCR assay design

Assays (TaqMan®) were designed using ABI Primer Express software, using sequences obtained from the NCBI database (www.ncbi.nlm.nih.gov). The assays for CYLV and CtCV-1 were designed during FV 382a. The assay for CaTV was designed and validated under a Defra funded PhD and is currently submitted for publication (Zurine Rozado, personal communication).

1.3. Real-time PCR

Real-time PCR (TaqMan®) was performed using generic conditions, essentially as described previously (Mumford *et al*., 2000), using TaqMan® core reagent kits (Applied Biosystems; Cat. No. 430 4441). Primers are used at a working concentration of 300pM and probes at 100pM, in each 25µl reaction. Assays were run on Applied Biosystems (ABI) 7900 machines.

1.4. Machine program for a RNA template:

30min at 48°C, 10min at 95°C, then 40 cycles of 95°C for 15sec and 60°C for 1min.

2. Practical work

2.1 *Aphid transmission (Grow in absence of vectors)*

Eighteen species of aphid are listed as pests on carrot (Blackman 2010; Blackman and Eastop 2000). The aim was to collect and test the UK species which are most likely to transmit viruses to and between carrot plants:

- *Cavariella aegopodii* (Willow-carrot aphid) – Common in the UK and known to transmit persistent and semi-persistent viruses to carrot.
- *C. pastinacae* – Common in the UK, colonises Apiaceae (previously known as Umbelliferae). Known to transmit viruses.
- *C. theobaldi* - Common in the UK, colonises Apiaceae. Known to transmit viruses.
- *Myzus persicae* (Peach potato aphid) - Extremely polyphagous and known to transmit over 100 viruses, including several persistent viruses.

Two different virus transmission tests were run for three clones of each species to be tested (if available) both following a standard method (after Verbeek *et al*, 2010) with only the exposure times to the infector plant varying.

Due to difficulties in culturing *C. pastinaceae* this was not used in the transmission experiments. The main transmission work was carried out using *Myzus persicae* and *Cavariella aegopodii* only. Three clones of each aphid species were used in the transmission work. 50 individuals of each clone of species of aphid were allowed to feed on a plant infected with CYLV virus, these were then transferred individually onto uninfected potential host plants and allowed to transmit the virus for 24 hours. The host plants used were: Carrot (*Daucus carota*); *Nicotiana bethamiana*, a tobacco species which is considered a 'common receiver' and is used as a standard bioassay plant in virology laboratory studies; and Chervil (*Anthriscus cerefolium*) was also used as a potential alternate host from the apiaceae.

The efficiency of transmission is then measured by the percentage of plants infected. Three replicates were carried out for each clone tested. The infection status of each plant was determined by testing plants by real-time RT PCR 28 days after the transmission test. This method was also repeated for CaTV.

2.1.1 Demonstration of Koch's Postulates

To demonstrate Koch's postulates, the above transmission tests were to be repeated in the opposite direction with the clone of each species identified as the most efficient vector being used. However, due to the failure to transmit CYLV for a large part of the project this was not possible within the project.

Plants found to be infected with CaTV following aphid transmission were allowed to grow on in the glasshouse for 6 months. In total 72 plants were grown on. At harvest 42 roots were cut and assessed for the presence of internal symptoms. All harvested roots were then cold stored at 4^{O}C for 14 days before being cut and assessed for internal symptom development.

2.2. Prevalence of carrot viruses in common field weeds (Grow in absence of virus reservoirs)

2.2.1. Weed samples collected during FV 382a (2012)

During the previous carrot virus work 90 samples of apiacious weeds from 4 different species were collected. These had been tested for the CMD viruses (CtRLV, CMoV and CtRLaVRNA) and PYFV. These samples were further tested for CYLV, CtCV-1 and CaTV.

2.2.2. Weed samples collected at Warwick Crop Centre 2010-1012

RNA extracts from apiaceous weed samples collected from around the UK between 2010 and 2012 were collected from Warwick Crop Centre. In total over 1500 sample extracts were collected. These had been tested previously by The Late Dez Barbara for the presence of CtRLV, PYFV and *Anthriscus yellows virus*.

The samples from 2010 were not RNA extracts, but were cDNA, a synthesized copy of positive RNA. Therefore these samples could not be tested for other viruses.

Sample extracts from 2011 and 2012 were tested for the presence of cytochrome oxidase (COX). This is a protein found in eukaryotes and the COX PCR test is used as a measure of

the quality of nucleic acid extraction. Nearly 1200 sample extracts were of sufficient quality for further virus testing. These samples were tested for CYLV, CtCV-1 and CaTV.

2.3. Distribution of the viruses in the UK (Isolate from similar crops)

2.3.1. Geographic distribution: Field testing

During 2014 three fields close to Fera (See Table 1.) were sampled in a 100mx100m grid pattern. Samples were picked at random at 10m intervals, giving 120 samples per field. These fields were sampled in July, and again in September to ascertain if there were any differences in virus content between the two testing dates. Additionally samples were taken of 120 carrot roots per field following the same sampling pattern. These were subsequently cut and assessed for the presence of internal necrosis.

Table 1. Field testing 2014 Field Sources

Field ID.	**Location**	**Sampled**
Sand Hutton	N. Yorks	July
Strensall	N. Yorks	July
Sutton on the Forest	N. Yorks	July

During 2015 (Table 2) three fields in North Yorkshire were sampled again according to the 100m grid pattern used in 2014. As this area was known to harbour the novel carrot viruses these fields were used as ‘control fields’. Additionally, three fields were sampled in Norfolk to represent a carrot growing area geographically remote from North Yorkshire to ascertain whether the novel viruses were limited to certain geographic areas.

Table 2. Field testing 2015 Source fields:

Field ID.	**Location**	**Date Planted**	**Sampled**
Stamford Bridge (Early)	N. Yorks	21-Feb	May
Stamford bridge (Late)	N. Yorks	10-Mar	August
Sutton on the Forest	N. Yorks	02-Mar	June
Elveden estate	Norfolk	Unknown	July
Highman estate "infected"	Norfolk	Unknown	July
Highman estate "healthy"	Norfolk	Unknown	July

In both cases samples were tested for the presence of the Carrot Motley Dwarf viruses, *Parsnip yellow fleck virus*, *Carrot yellow leaf virus*, Carrot closterovirus-1 and Carrot torrado virus. Additionally any carrot samples submitted by agronomists during the period covered by the project were also tested for the full suite of carrot viruses. 22 samples were submitted from UK carrot fields including crops grown in Lancashire and the West Midlands. A further 11 samples were submitted through contacts in the EU, with samples originating from Denmark and The Netherlands.

2.4. Seed testing (Plant clean seed)

Samples of carrot seed were retained following other seed testing activities. In total 10 seed lots were tested and in each case 1500 seeds were tested. These were sub-sampled into 30

sub-samples of 50 seeds each. Extraction and testing was carried out in accordance with the diagnostics section above.

Results

3.1. Aphid Transmission of carrot viruses

For each virus (CYLV and CaTV), 3 clones of each species were used or the transmission work, giving a total of 900 plants per virus species tested. Additionally for CYLV a reduced number of clones of *C. pastinaceae* and *C. theobaldi* were also used in transmission studies. CYLV was observed to transmit at a very low efficiency (Table 3) but in the three vector species where transmission occurred transmission from weeds into carrots was achieved at a slightly higher rate than transmission between carrots. This transmission was only achieved at the very end of the project and there were not sufficient numbers of infected carrots to complete the demonstration of Koch's Postulates. Because Koch's postulates could not be demonstrated for CYLV as the causal agent of internal necrosis, this causal relationship remains an association between presence of virus and the symptom in the roots.

Table 3. Transmission rates of CYLV with three aphid vector species. 'Weed' samples were field sourced infected cow parsley (*Anthriscus sylvestris*)

Vector Species	Transmission experiment	% Transmission
Myzus Persicae	Weed - Carrot	0.2%
	Carrot - Carrot	0.0%
C. aegopodii	Weed - Carrot	0.5%
	Carrot - Carrot	0.2%
C. theobaldi	Weed - Carrot	0.5%
	Carrot - Carrot	0.0%

Figure 2. Symptoms of CaTV infection in Chervil infected though aphid transmission. Chervil plant showing interveinal chlorosis.

The transmission of CaTV however was successful with both the carrot aphid (*C. aegopodii*) and the peach-potato aphid (*M. persicae*). The symptoms of CaTV on an infected chervil test plant can be seen in Figure 2. The observed relative rate of transmission of the two aphid species can be seen in Table 4 (a) and (b). *M. persicae* (Table 4a) was observed to be a more efficient vector of CaTV than *C. aegopodii* (Table 4b) across all test plant species. These data should not be considered to be an absolute measure of efficiency between plant species as the rate of infection may be affected by relative susceptibility of each species to the virus.

Table 4 (a) and (b). Transmission of CaTV to Carrot, Chervil and a tobacco, *Nicotiana benthamiana*.

(a) Transmission of CaTV by *Myzus persicae* (Peach-Potato aphid)

	Positive	Negative	%infection
N. benthamiana	19	130	12.7
Carrot	15	135	10
Chervil	53	95	35.3

(b) Transmission of CaTV by *Cavariella aegopodii* (Willow-Carrot aphid)

	Positive	Negative	%infection
N. benthamiana	0	150	0
Carrot	4	146	2.7
Chervil	8	141	5.3

In total, including plants infected through exploratory transmission work, 73 carrot plants were infected with CaTV. These were grown on in the glasshouse for 6 months (4 inch pots with potting compost; day length 16 hours; temperature 16-18C). During growth they were assessed for the development of virus symptoms. Due to heavy infection with powdery mildew and the stress of being grown in glasshouse pots there were no definitive foliar symptoms. At harvest 42 of these roots were cut and assessed for internal symptoms. Five roots showed signs of internal discolouration near the crown, a single root exhibiting symptoms consistent with viral root necrosis (See Figure 3). Roots were then cold-stored for 14 days and then cut and re-assessed for internal symptoms. In total eight (8) roots showed some sign of internal discolouration, with only the single root again showing symptoms consistent with viral root

necrosis. All roots were subsequently retested for the presence of Carrot yellow leaf virus, Carrot torrado virus and Carrot closterovirus-1. In each case the roots were negative for the viruses.

Figure 3. Carrot root following aphid infection with CaTV exhibiting symptoms consistent with root necrosis.

3.2. Weed Testing

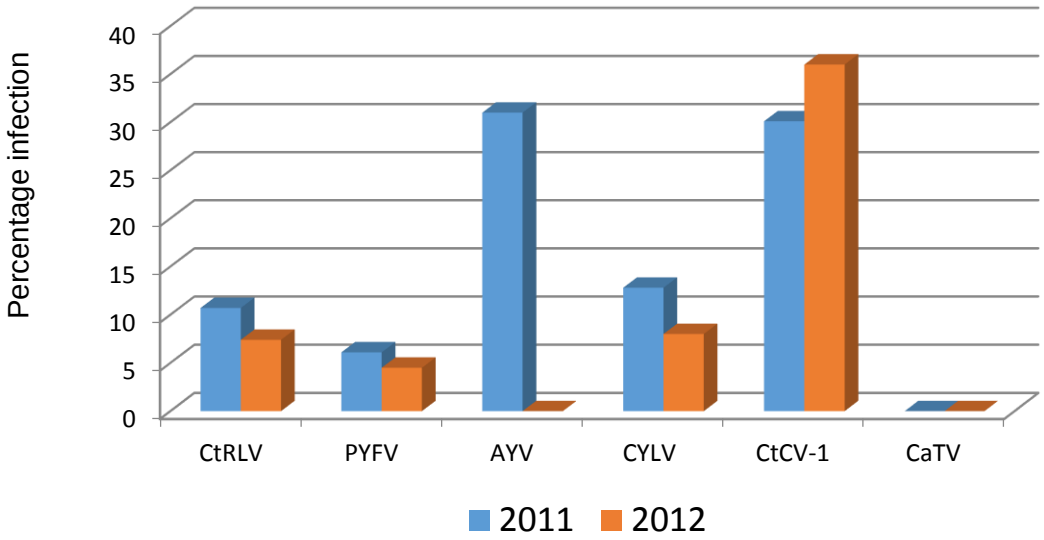
During 2012, weed samples had been collected under project FV 382a and these had been previously tested for the CMD viruses (CtRLV, CMoV and CtRLaVRNA) and PYFV. Using assays described above these samples were tested for CYLV, CtCV-1 and CaTV (Table 5). From these limited data the CMD complex of viruses were present in hogweed, cow parsley and rough chervil. However CYLV and the similar virus CtCV-1 were only found to be infecting cow parsley. CtCV-1 was the highest prevalence virus in these samples present in nearly 30% of cow parsley plants tested. Carrot torrado virus was not found to be present in any of the weed samples tested from Fera sampling.

Table 5. Results of testing Fera collected in 2012 weed samples during FV 382a for the presence of the novel viruses CYLV, CaTV and CtCV. These samples had been previously tested under project FV 382a for CMD viruses and PYFV.

Species	No. of fields sampled	No. Of Samples	CtRLV	CMoV	CtRLa VRNA	PYFV	CYLV	CtCV-1	CaTV
Hogweed	9	59	1 (1.7%)	1 (1.7%)		1 (1.7%)			
Cow Parsley	7	27	7 (25.9%)				3 (11.1%)	8 (29.6%)	
Hemlock	1	1							
Rough Chervil	2	3	1 (33%)						
Total	9	90	9	1	0	1	3	8	0

Weed samples extracts from Warwick crop centre from 2011 and 2012 were tested for the presence of CYLV, CtCV-1 and CaTV. These samples had been previously tested for CMD viruses and PYFV. In total 1112 extracts were quality checked for COX. After removing extracts which did not meet quality criteria, and any extracts where comparable data were not available for the CMD virus and PYFV, 938 sample extracts were tested for virus infection (478 samples from 2011, 460 samples from 2012).

Figure 4. Summary results from testing Warwick Crop Centre apiaceous weed samples collected 2011and 2012. Results are presented for virus findings as a percentage of plants tested in each year. Results for CtRLV, PYFV and AYV are from the original work carried out at Warwick. Results from CYLV, CtCV-1 and CaTV are from Fera testing under this project.

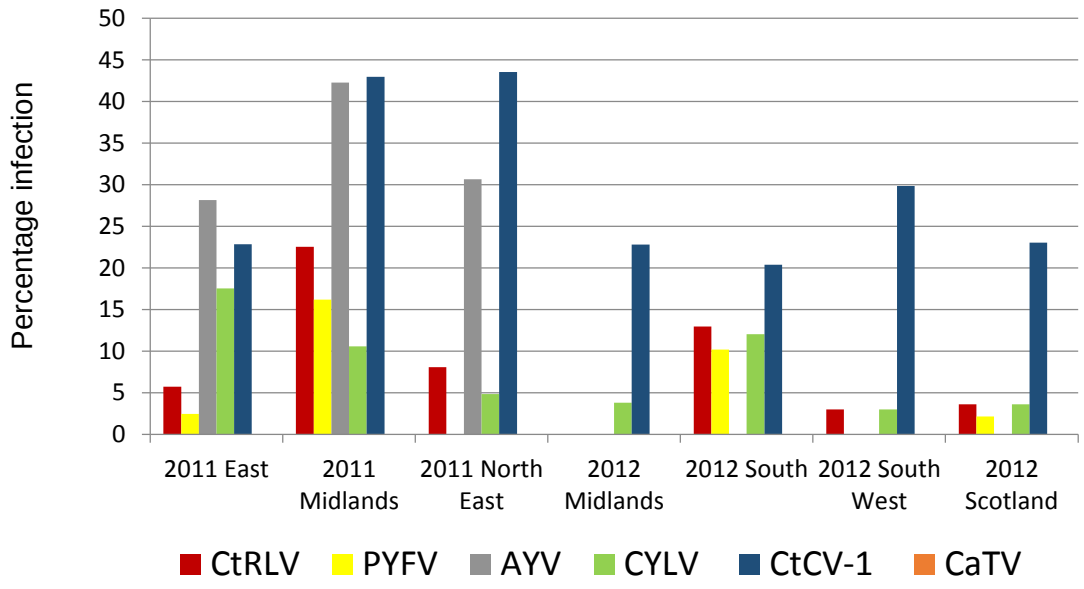


A summary of the findings for each virus can be seen in Figure 4. In each case the levels of virus detected in each year were comparable, with the exception of *Anthriscus yellows virus,* which was the most commonly detected virus in 2011 samples, but absent from the 2012 sample set. Although this virus does not directly affect carrot it is essential for the transmission of PYFV. Carrot closterovirus-1 was almost as commonly detected in 2011, and overall was the most commonly detected virus present in 29% and 35% of samples in 2011 and 2012 respectivley. *Carrot yellow leaf virus* was present in 8-12% of samples (2012-2011 respectively). Again CaTV was absent from all samples tested.

The sample results broken down by geographic origin are presented in Figure 5. Carrot closterovirus-1 was the most prevalent virus in every region, with the exception of samples taken in the East of England (Norfolk and Peterborough area) during 2011. The virus was detected from at least 20% of weeds in all areas in both years, and at considerably higher incidences in the Midlands (West Midlands/Warwickshire) and North East (North Yorkshire) in

2011 and in the South West in 2012. *Carrot yellow leaf virus* was the only other virus detected consistently across all areas in both years, with greatest prevalence in the East(2011) and the South (2012). Generally speaking the prevalence of this virus in weeds increased the further south and east in the country. These data give strong evidence that for the two closteroviruses (CYLV and CtCV-1) detected in FV 382a have a national distribution in the UK.

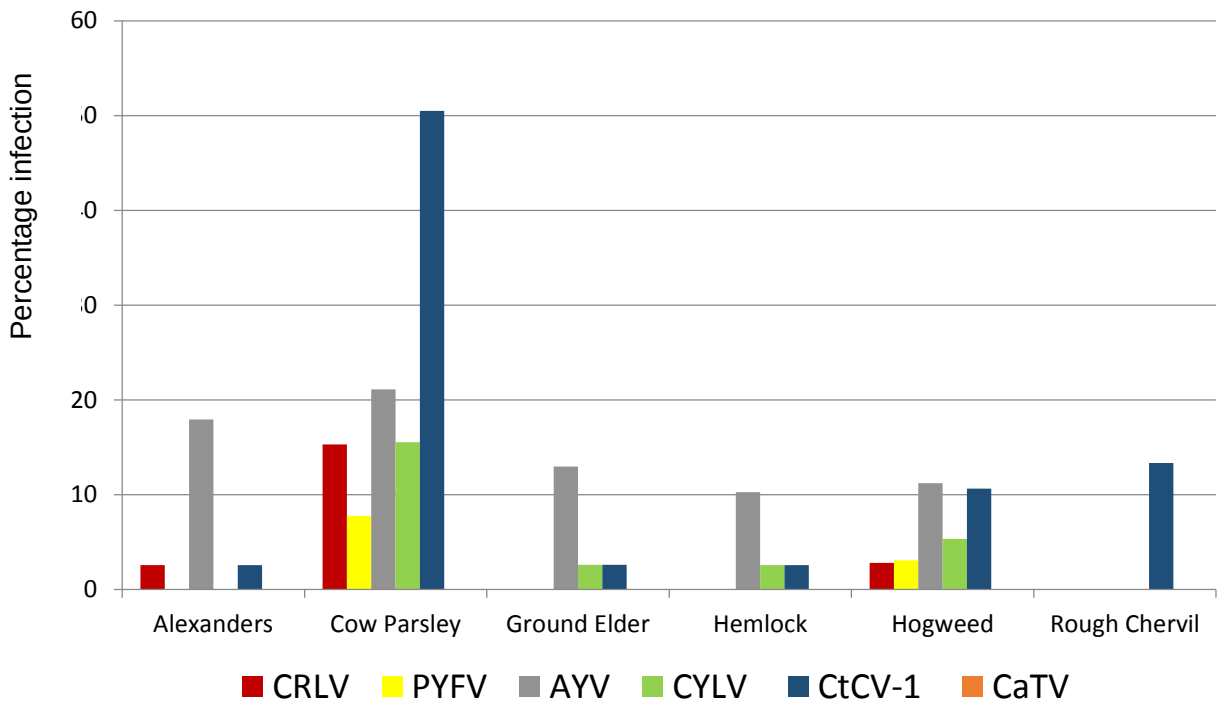
Figure 5. Proportion of virus findings from Warwick Crop Centre apiaceous weed samples collected 2011and 2012 by geographic origin. Results for CtRLV, PYFV and AYV are from the original work carried out at Warwick. Results from CYLV, CtCV-1 and CaTV are from Fera testing under this project.



The sample results from the Warwick collected weed samples are presented in Figure 6. Cow parsley (*Anthriscus sylvestris*) was the most commonly infected species with samples infected with samples infected with every virus except CaTV, which was absent. Half of all cow parsley plants tested were infected with CtCV-1, and there was evidence of this virus infecting the other species tested as well. Cow parsley was also the host most commonly infected with CYLV. This virus was also detected from hogweed (*Heracleum sphondylium*), ground elder

(*Aegopodium podagraria*) and hemlock (*Conium maculatum*), with the latter two species being new host records for this virus.

Figure 6. Proportion of virus findings from Warwick Crop Centre apiaceous weed samples collected 2011and 2012 by species sampled. Results for CtRLV, PYFV and AYV are from the original work carried out at Warwick. Results from CYLV, CtCV-1 and CaTV are from Fera testing under this project.



3.3. Distribution in carrot crops

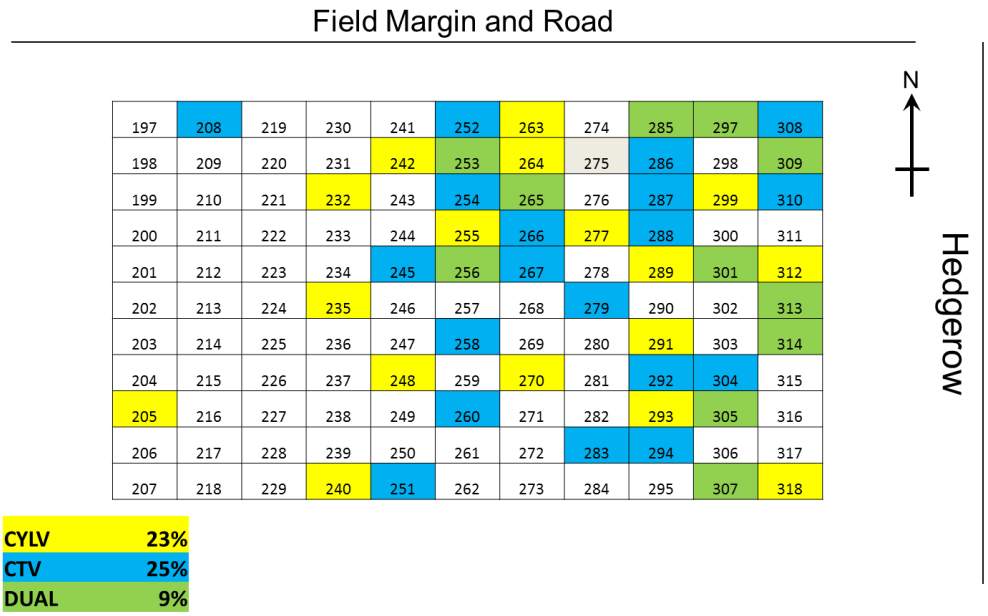
3.3.1 Field testing 2014

To ascertain the optimum sampling strategy for the novel viruses detected in FV 382a fields close to Fera were selected from prior knowledge of fields likely to be badly affected by viruses (Howard Hinds, pers.comm.). These were then visited on a regular basis throughout the season to check for development of yellowing and to test for the presence of detectable virus. There was a high incidence of plants observed with strong interveinal chlorosis (Figure 7). Unfortunately due to the high incidence of multiple virus infections, few inferences could be made about the relationship between individual viruses and observable symptoms.

Figure 7. An example of carrot leaf with yellowing symptoms associated with non-specific viral infections.

Fields were staked out to give measured plots 100m by 100m. samples picked at random at regular distance (10m). For 2014 samples the presence and distribution of virus infected plants within the field plots were plotted to look at within-field distribution (e.g. Figure 8). Although these results did not show any conclusive distribution of viruses within plots, the incidence of these viruses was observed to increase towards the field margins and hedgerows.

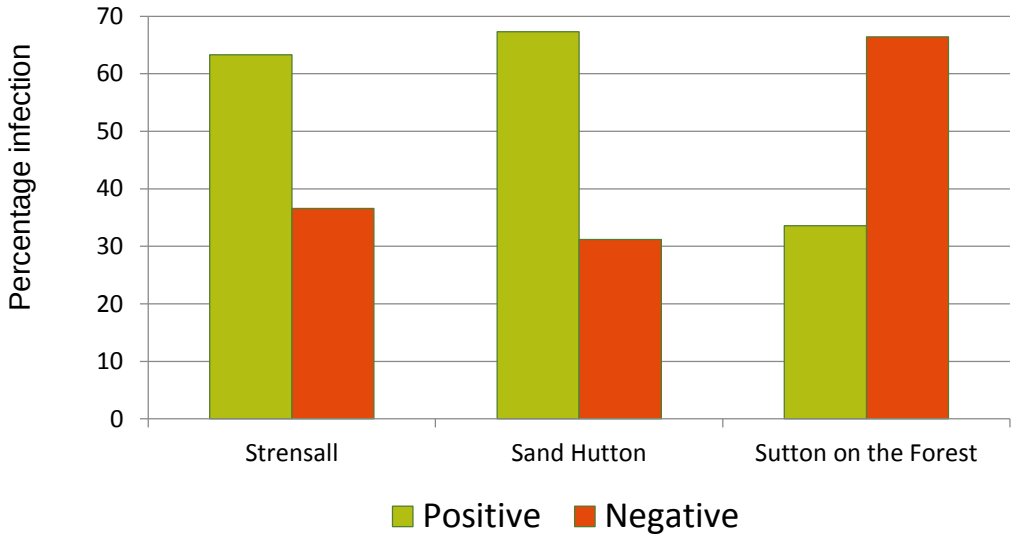
Figure 8. Distribution of CYLV and CaTV in High Roans Field, Strensall, North Yorkshire.



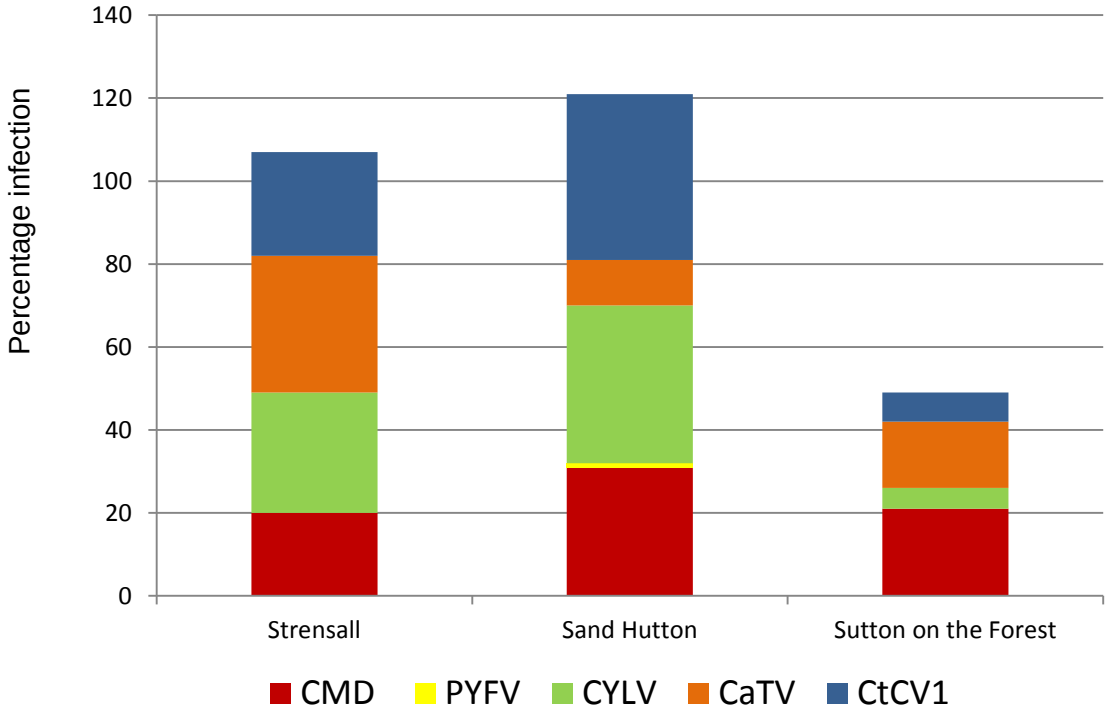
There was a high incidence of virus recorded across all three fields in 2014 (Figure 9a). Two fields (Strensall and Sand Hutton) had a markedly higher virus incidence than was detected in the third field (Sutton on the Forest). The percentage of each virus found is presented in Figure 9b.

Figure 9. Percentage virus content of fields intensively sampled during 2014 field work. (a) Data presented as percentage of total plants showing infection, and (b) Total percentage of virus content. As CMD is a complex of viruses dependent upon CtRLV for transmission the presence of any of these viruses in a plant is counted as a single case of CMD.

(a)



(b)

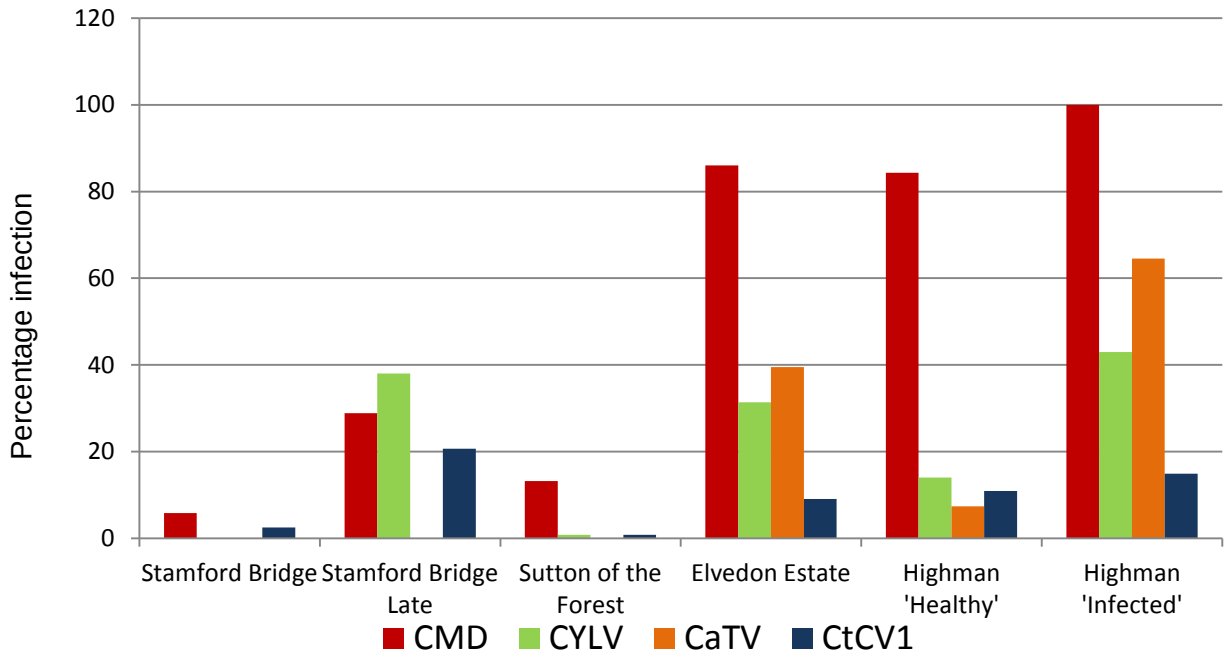


The incidence of CMD viruses was calculated by taking the presence of at least one virus of this complex as evidence of CMD infection. As this is a complex of three viruses, including each virus individually in the data presentation would skew the findings to appear that these interdependent viruses were present in a higher proportion than other viruses. The largest proportion of this drop in virus incidence was a reduction in the two closteroviruses (CYLV and CtCV-1). The high incidence of viruses which had been previously overlooked such as CYLV, or unknown (CaTV and CtCV-1) is evident in all fields. *Parsnip yellow fleck virus* was largely absent from the field samples collected in 2014.

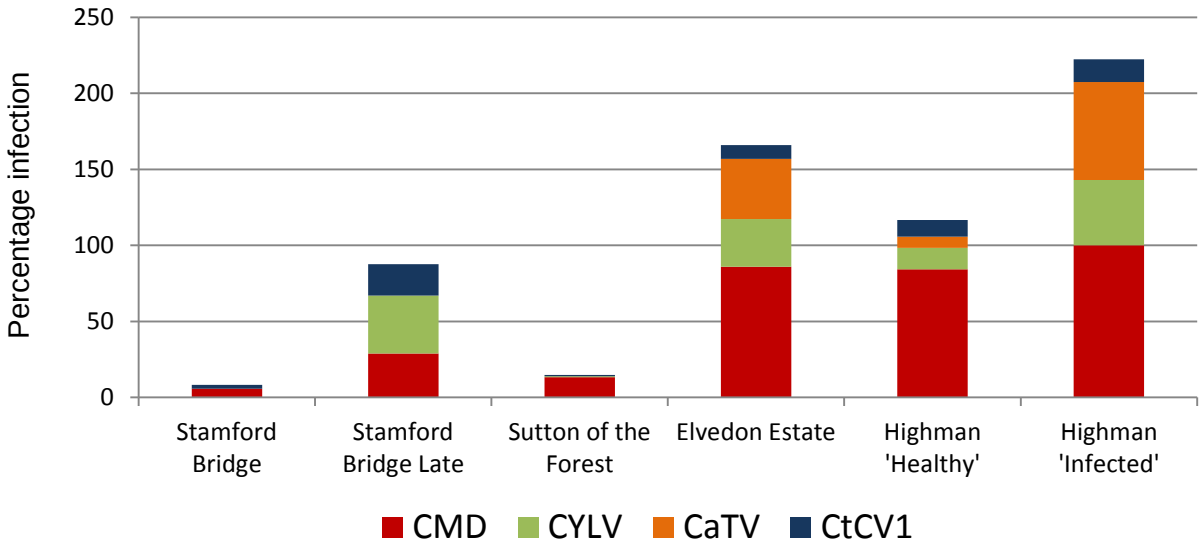
3.3.2 Field testing 2015

Figure 10. Percentage virus content of fields intensively sampled during 2015 field work. (a) Data presented as percentage of total plants infected, and (b) Total percentage of virus content.

(a)



(b)



All fields in 2015 were sampled using the plot sampling method developed in 2014. Viruses of the Carrot motley dwarf complex were present in all fields but at greater incidences in the late sampled Yorkshire field and in the fields in Norfolk. In one field there was evidence of CMD infection in every plant tested (Highman Estate, Infected). Carrot torrado virus was absent in all the Yorkshire fields sampled. *Carrot yellow leaf virus* and Carrot closterovirus-1 were present in Yorkshire only in the late sampled fields.

3.3.3. General diagnostic samples

During the project carrot samples submitted to the Fera Plant Clinic were also tested for the presence of carrot viruses (Table 6). The results of this testing are presented as contribution in kind and as the samples were not tested directly under this project the origin of these samples is subject to commercial confidentiality. In total 33 carrot samples were tested from a range of sources and nearly half of the samples were found to be infected with one or more viruses. 10 samples were found to contain multiple viruses, in 3 cases these were solely the CMD viruses. The EU samples were submitted from the Netherlands and Denmark demonstrating that the novel viruses are present in mainland Europe as well as the UK.

Table 6. Results from testing carrot samples submitted to the laboratory over the course of the project.

Sample Origin	No. of Samples	CtRLV	CMoV	CTRLaVRNA	PYFV	CYLV	CaTV	CtCV	Negative
EU	11	3	3	2	0	3	1	1	5
UK	22	4	3	4	1	4	4	3	11
Total	33	7	6	6	1	7	5	4	16

3.4 Seed Testing

Seed lots were screened for the presence of CaTV only. Of the 10 seed lots tested only 2 were found to contain the virus (Table 7). The virus was found in a low number of bulks. Interpretation of virus content was carried out using the ISTA programme SeedCalc8. This calculated the mean virus content from these bulks to be 0.29% and 0.53% respectively.

Table 7. Results from testing seed lots for the presence of CaTV.

Sample number	No. of bulks positive	Calculated mean % virus
Lot 1	4 / 30	0.29
Lot 2	7 / 30	0.53

Discussion

Carrot yellow leaf virus has been recognised in the UK for over 30 years (Bem and Murant, 1979), but the distribution and incidence of this virus in the UK was unknown. Although the virus is recorded as being both aphid transmitted and mechanically transmissible (Dijk and Bos, 1991) the virus was previously reported to be of low transmissibility and back transmission to carrot had not been successfully demonstrated by either transmission method. The primary objective of this project was to demonstrate aphid transmission of CYLV and to try to experimentally induce root necrosis through a growth cycle of artificially infected carrots. Attempting aphid transmission with a virus that is phloem limited and poorly transmissible was known to have a limited chance of success, a task made more challenging by working with source inoculum from carrot and weed samples taken from the field where most samples were infected with multiple viruses and did not survive well after being uprooted from the field. This involved field visits to identify potentially infected hosts plants, testing samples from these

plants in the laboratory and then returning to the field to collect plants with known infections. Infector plants were then used to rear aphid populations before transferring individual infected aphids onto seedlings in trays. Despite several rounds of this work transmission was only achieved at a very low level (see Table 3). Given that these data for CYLV are the culmination of 900 individual aphid transmission events, these low levels of transmission support the lack of prior data in the literature where only limited numbers of aphids were used for transmission experiments.

Due to the exploratory nature of transmission work reported here there is a distinct possibility that transmission work was not carried out under optimum environmental conditions. It is known that temperature and relative humidity can affect the rate of virus transmission (e.g. Singh et al, 1988) and further work would be required to optimise conditions for any future virus transmission study. Despite this, this report represents the first successful experimental transmission of CYLV into carrot. Unfortunately the successful transmission of this virus occurred in the final month of the project and CYLV could not, therefore, be conclusively demonstrated CYLV as the cause of root necrosis. As a consequence of this limited success, other areas of study were brought forward to give value to the work, primarily to look at the distribution and field incidence of the novel carrot viruses in crops and alternate weed hosts.

At the same time as the CYLV transmission work was ongoing, the same team had further funding from the Defra Plant Health Capability funding stream to investigate the incidence and impact of Carrot torrado virus (presented here as contribution in kind). This work had greater success and through this work we were able to experimentally demonstrate aphid transmission of CaTV a virus which was first detected during the previous AHDB carrot virus project (FV 382a). As a novel virus, first detected through sequence analysis, further information on the biology, incidence and impact of this virus was crucial to make informed decisions on the plant health status of this finding. There were no obvious inferences that could be made on the foliar and root symptoms of CaTV due to the ‘unnatural’ growing conditions of the infected carrot plants, namely in pots in a glasshouse. These conditions

made the plants very susceptible to infections with powdery mildew badly affecting the foliage. All the plants showed excessive lateral root grown and root deformation. A small number of plants showed limited evidence of necrosis around the crown and a single root showed necrosis around the core, however, this is likely to be a result of several stress factors affecting the plant and cannot be definitively attributed to infection by CaTV. Other torradoviruses were first described affecting tomatoes and had been demonstrated to be transmitted by whiteflies (Amari et al, 2008; Verbeek et al, 2014a). However, there has also been a report of another virus from this genus affecting lettuce in northern Europe (Verbeek et al, 2014b). Genetically these non-tomato infecting torradoviruses cluster in a group distinct from the tomato infecting viruses of the genus and as whiteflies are not widely recognised as pests on field crops in northern Europe it was proposed that other vectors may play a role in the epidemiology of these viruses (van der Vlugt et al, 2015). This project has demonstrated for the first time that this virus is aphid transmissible by both the willow carrot aphid (*Cavariella aegopodii*) and the peach-potato aphid (*Myzus persicae*). Additionally the demonstration of weed to carrot transmission of CYLV by these same vector species has important consequences for virus management programmes. In carrot most programmes focus on control of willow-carrot aphid alone. Additionally, the potential for *M. persicae* to vector viruses in carrots also means that aphicide resistance must also now be considered as a factor in within aphid control programmes. It was noted in several affected fields that aphid control measures were not fully effective due to the presence of live aphids and shed skins.

Data gathered on incidence of novel carrot viruses from both weed sources and from carrot fields show that these viruses have a national and international distribution. These findings represent the first records of CaTV and CtCV-1 outside of the UK. These weed data also represent the several new host records for CYLV, CaTV and CtCV-1. Taken in conjunction with the aphid transmission work where chervil was also infected with CaTV, these novel viruses should also be considered as a potential risk to other apiaceous crops including herb species. CYLV and CtCV-1 are closely related viruses and as such there is potential that

CtCV-1 may have the potential to produce necrosis in an analogous way to that associated with CYLV. Both are closteroviruses, a group which are associated with inducing symptoms associated with vascular damage in other crops such as bark damage, stem pitting or leaf brittleness.

In the field setting cow parsley (*Anthriscus sylvestris*) appears to be the major non-crop host of all the carrot viruses. This may be due to relative susceptibility of this species to the different viruses or may be the result of relative attractiveness of this host to different aphid species. The two closteroviruses, CYLV and CtCV-1, were also the only viruses found in weeds from every region in both years with CtCV-1 being consistently among the viruses most commonly detected from the weed samples. It was of interest to note that CYLV was more commonly detected from carrot crops that CtCV-1, but CtCV-1 was more commonly detected in weed hosts. Further inferences on these relationships cannot be drawn without an investigation into the genetic relationships of these virus populations.

The one surprising result of the weed testing was the complete absence of CaTV. Given that the virus was experimentally transmitted to chervil, another species from the Apiaceae and also *Nicotiana benthamiana* a member of the Solanaceae, the virus has been demonstrated to infect species from a range of families. It is notable that the virus has not been detected from alternate hosts in the field. The weeds sampled here represent the 'usual suspects' for carrot virus field hosts. This leaves two potential explanations for CaTV epidemiology: either the virus has no alternate hosts and is circulating in carrot crops, or there is an alternate host outside the range tested here, possibly a non-apiaceous host.To demonstrate this latter possibility would require extensive testing of a broad range of weed and crop species with little evidence to support this course of testing. If CaTV is circulating in carrot crops this may be due to the virus being brought into crops in seed. The seed testing results presented here showed that CaTV has the potential to be seed transmitted, although this requires further investigation through a seed grow-out experiment to confirm that this virus is seed transmissible. Although a limited study, the proportion of seed lots found to be infected and

the incidence of virus within infected seed lots suggests that this may not be the sole source of virus within field crops. If the virus is found to be circulating in carrot crops this may be due to the agronomy of carrots in the UK. Since crops are in the ground year-round, last year's crop under straw may provide a source of virus for the subsequent crop's in the local area. This would be a similar epidemiology to that suggested by Dez Barbara for the CMD viruses (Barbara, 2011) and in both cases the recirculation of viruses from carrots stored under straw should not be discounted.

The testing of carrot field plots mirrored the high incidence of the novel viruses seen in the weed testing results. In both the weed testing and the carrot field testing there was a span of years covered, and though not a complete dataset covering all years and host sources, this gives a strong indication that the viruses may be variable in different regions from year to year.

There was a very low incidence of *Parsnip yellow fleck virus* recorded against a background of relatively high virus incidence in Yorkshire fields in 2014. This virus is known to sporadically occur in carrot crops, devastating in some years, but less so in others. This variation is, largely because the virus needs *Anthriscus yellows virus* to be present in cow parsley for acquisition of PYFV to occur. The weed data from 2011 and 2012 demonstrate the potential variability of this virus across years and regions. This low incidence may therefore be a result of a genuine low incidence as a result of low transmission. However, it is also possible that the low incidence in 2014 carrot crops may be the result of the biology of the virus. One of the consequences of PYFV infection early in the season is seedling death, if this has occurred then the virus would, in effect, be self-eliminating from the sample set and would not be present in diagnostic samples collected later in the season. There was a marked difference in virus health in one of the three 2014 crops. All three crops were from the same grower and therefore had been subjected to similar agronomic practices as the other crops. Apart from geographic location the main difference between this and the other two crops was a later planting date. This later planting may have reduced exposure to virus infection pressure in the early season, though the effect of planting date would require further investigation. It was also interesting to

note that the relative incidence of CtCV-1 was much greater in weeds than the very similar CYLV, however in carrot fields the incidence of these two viruses was similar. This could be a consequence of differences in vector transmissibility or could be due to relative virus susceptibility between carrots and wild apiaceous hosts.

In overview of the field and weed data the expected North-South increase was observed. Crops tend to be planted earlier in the south and east of the country, where aphid migrations also tend to occur earlier and in greater numbers. This has the effect of exposing crops to a higher potential to infection from aphid virus vectors for longer in the season.

The main questions that remain to be answered on the epidemiology of the novel viruses are the role of alternate hosts (weeds) as virus sources, and the importance of seed as a potential infection route. Previous findings on CtRLV (Barbara, 2011) suggested there were different populations of CtRLV in weeds compared to the populations from carrot, indicating that the source of carrot virus infections was from carrots and not from weeds. As suggested above reinfection of new-season carrot crops from crops under straw is also a potential epidemiological model for CaTV. These findings could have serious implications for virus management. To confirm the relative importance of weeds in the epidemiology of CYLV and CtCV-1 in depth sequencing work will be needed to follow up this previous population genetics work. At this time limited inferences can be made about the role of various virus sources across weeds and carrots. Additionally, within this project seed lots have been shown to carry CaTV, and from current laboratory testing in support of export there is evidence of seed lots being contaminated with carrot viruses including CtRLV and the other viruses from the CMD complex (Data not presented). The role of seed sources within the epidemiology of carrot virus is currently an unknown quantity. For viruses where there is no obvious weed source (CaTV), and for those where there is evidence to suggest weeds do not play a part in their epidemiology (CtRLV), seed sources should be a focus for future investigative work. However, the recommendations from previous work by Barbara (2011) should also be considered; limiting

the exposure to new season crops from crops stored in the ground will help to minimise this potential source of inoculum.

It is difficult to know the precise impacts of these viruses on the carrot crops investigated within this project. With the exception of carrots which were tested and harvested early in the season, and had consequently low levels of virus infection, other crops were heavily infected. Plants were infected with multiple viruses; both complexes such as carrot motley dwarf complex, and also the other viruses included in the suite of testing. This makes inferences about the effect of individual viruses difficult to draw out. Additionally the cumulative impact of multiple virus infections remains unknown. Given that a chlorotic virus infection effectively reduces the leaf area available for photosynthesis by the reduction in 'green' foliage, it is likely that there will be a reduction in yield and potentially a reduction in quality in infected carrots. However, to investigate this would require a comparative trial between infected and uninfected crops. This could possibly carried out as part of a future trial on control measures e.g. a spray efficacy/IPM trial.

The emergence of *M. persicae* as well as other *Cavariella* species as potential virus vectors in carrot crops increases the understanding of virus epidemics in seasons where large migrations of willow-carrot aphid don't occur. *M.Persicae* entering carrot fields are likely to be aphicide resistant. Currently the resistance status of willow-carrot aphid (*Cavariella aegopodii*) is unknown, and this is an area in clear need of further investigation. With a limited range of chemical control options available to the grower alternative management methods should be considered including barrier methods such as using fleece on crops in high risk areas; and IPM approaches to increase natural predators. Additionally to help protect the industry in the longer term varieties with good virus resistance, or at the very least, symptom tolerance should be investigated as part of breeding programmes to minimise the impacts of virus infections.

Conclusions

If we reconsider the fundamental principles of plant virus management in the light of these new data we can now say:

- Plant clean seed: There is a potential that carrot seed may be a source of CaTV
- Grow in absence of vectors: *Myzus persicae* must now be considered as a vector of carrot viruses along with other *Cavariella* species including *C. aegopodii*.
- Grow in absence of virus reservoirs: Both common weed hosts and carrot crops may form a source of virus infections into uninfected crops.
- Isolate from similar crops: The separation of crops in this respect could be isolation from other carrot crops 'in time' as well as geographic isolation.
- Use resistant (or tolerant?) varieties: There is still limited data on the susceptibility of a range of varieties. The majority of work reported here has been carried out on cv. Nairobi as this is the variety most commonly grown in the UK.

Although CYLV could not be demonstrated as causing necrosis within this project, the virus has been shown to aphid transmitted. Work related to this project has shown that the virus CaTV is transmitted by aphids, a first demonstration of aphid transmission for a torradovirus. The novel viruses CtCV-1 and CaTV, as well as CYLV appear to have a national distribution in both field crops and alternate host sources, giving potential for transmission into carrot crops as well as other members of the apiaceae, such as field grown herbs. They also appear to be present in carrot fields in Europe and may therefore be of international importance in carrot production. The precise role of weeds in the epidemiology of these viruses remains to be confirmed, however, they may play an important role as sources of CYLV and CtCV-1. From these data weeds appear to be of limited significance as a source of CaTV infections. The finding of greatest significance for growers is the demonstration of *Myzus persicae* as a

potential vector in carrots. This will have implications for aphid control strategies as this aphid species has multiple aphicide resistance mechanisms and will present major challenges for season long control of aphids.

Knowledge and Technology Transfer

Presentations:

1. Society of General Microbiology Annual Conference, Liverpool, UK. 14-17 April 2014. *Carrot yellow leaf virus is associated with internal root necrosis*, Presentation on FV 382a and forward look of FV 382b.
2. ISHS conference on carrots and apiaceous crops, Anger, France.17-19 September 2014. *Carrot yellow leaf virus is associated with internal root necrosis*, Presentation on FV 382a and initial field results of FV 382b.
3. Syngenta Growers Meeting, Newark, UK. 26 February 2015. *Carrot virus update*. Presentation on FV 382a and forward look of FV 382b.
4. British Carrot Growers Association Spring Technical Meeting, Peterborough, UK. 26 March 2015. *Everything you ever wanted to know about carrot viruses but were afraid to ask…* Presentation on FV 382a and interim results of FV 382b.
5. Meeting of the International Working Group on Legume and Vegetable Viruses, Haarlem, Netherlands. 31 August-3 September 2015. *Carrot yellow leaf virus is associated with internal root necrosis.* Presentation on FV 382a and interim results of FV 382b.

Posters:

1. Flint L, Barbara D, Clarkson J, Skelton A, Fox A. (2015) *Are umbelliferous weeds the source of carrot virus epidemics? Presented at:* The International Working Group on Legume and Vegetable Viruses, Haarlem, Netherlands. 31 August-3 September 2015. (Forward look overview of umbelliferous weed testing carried out under FV 382b).

Scientific Publications

1. Adams IP, Skelton A, Macarthur R, Hodges T, Hinds H, Flint L, Deb Nath P, Boonham N, Fox A. (2014) *Carrot yellow leaf virus* Is Associated with Carrot Internal Necrosis. *PLoS ONE* 9(11): e109125. doi:10.1371/journal.pone.0109125
(Paper summarising FV 382a)

Grey Literature

2. *Carrot viruses in Great Britain: Current Perspectives*. Vegetable Yearbook and Buyers Guide 2104, ACT Publishing (Overview of current knowledge including FV 382a and forward look to FV 382b)

Overview of carrot viruses

Virus names are only written in italicised script once they are formally recognised by the International committee on Taxonomy of Viruses (ICTV). Generally speaking plant viruses are named using the following convention:

Common name of initial host – Symptom observed – Virus

This can lead to some confusion if a virus has multiple host species e.g. Cucumber mosaic virus has over 1200 known hosts; or when a symptom is idiosyncratic to a particular variety or is a temporary reaction. Virus nomenclature has been further complicated by the use of novel sequencing techniques, such as those used in FV 382a, where previously unknown viruses are discovered with no direct reference to symptomatic context. In these cases the virus is named after the genus level to which it can be assigned. If the virus is from a novel genus, then it is named after the 'new' genus name.

The information below is designed to give an overview of the viruses referred to in this report.

Parsnip yellow fleck virus **(PYFV)**

PYFV is important as an early season disease where it is associated with seedling death (see Figure 11). The virus requires *Anthriscus yellows virus* (AYV) for transmission, and this second virus provides a 'molecular glue' to enable the PYFV to be retained within the aphid foregut. The aphid can be thought of as a 'flying syringe' drawing up the virus and then passing it on through subsequent feeding activity. Transmission is rapid, typically taking less than a few minutes to pass on the virus. The main vector of this virus is considered to be *Cavariella aegopodii*, the willow-carrot aphid.

Figure 11. Seedling death in carrots caused *Parsnip yellow fleck virus*

AHDB-Horticulture project FV 228a demonstrated that the source of PYFV infections in carrots are most likely to be associated with cow parsley (*Anthriscus sylvestris*), a common hedgerow weed. As carrots are not a host of AYV, once they are infected with *Parsnip yellow fleck virus* the virus cannot be passed on.

In many seasons, PYFV does not commonly occur. The reasons for this sporadic occurrence are still unknown, but it is possibly due to its complicated epidemiology involving AYV, which limits onward spread in carrot crops. Work conducted at Warwick Crop Centre suggested a close relationship between observed symptom, root weight and the proportion of plants infected with PYFV suggesting that this virus can cause stunting in mature carrot crops (Dez Barbara, pers. comm.).

Carrot Motley Dwarf disease (CMD)

Carrot Motley Dwarf (CMD) is a disease complex comprising of *Carrot red leaf virus* (CtRLV), *Carrot mottle virus* (CMoV) and Carrot red leaf associated viral RNA (CtRLVaRNA). The disease complex can only be transmitted if CtRLV is present as the other two viral components are enclosed within the CtRLV virus particle during aphid acquisition and transmission. However, the individual component pathogens can be found in single infections. The virus is taken up by the aphid and passes through the gut and into the salivary gland where it can be passed on through feeding activity. This process can take several hours. Carrot infections are thought to originate from other carrots rather than weed hosts.

Figure 12. Leaf reddening and dwarfing caused by infection with CMD disease complex

CtRLV is associated with leaf reddening (See Figure 12) and CMoV with mottling which is a dappled yellowing of the leaf. However, in experimental studies, single infections by either of these viruses resulted in mild symptoms. The two viruses in co-infection have a greater effect

on the plant and the result is called carrot motley dwarf disease. The third virus in the complex, CtRLVaRNA, is not known to have any noticeable effect on disease symptoms.

While foliar symptoms may be obvious, there is little data on root symptoms or crop loss due to these viruses. Anecdotally infections with CMD have been linked to root symptoms such as excessive lateral root hairs (bearding) and root cracks and splits (splitting or kippering). Visual identification of this disease complex is not helped by leaf reddening etc. being a general response to stress or physical damage and there are also similar symptoms caused by infections with other pathogens such as phytoplasmas.

Carrot yellow leaf virus (CYLV)

Although this virus has been known to occur in the UK since 1980, very little research was conducted into the virus as it was considered a minor issue. However, FV 382a demonstrated that this virus was strongly associated with carrot internal necrosis (Figure 13).

Figure 13. Carrot root cross section showing presence of internal necrosis associated with infection from CYLV

The virus is known to be transmitted by a similar mechanism to PYFV, where the virus is sucked into the foregut of the aphid and can be rapidly transmitted into a new host. Unlike PYFV, *Carrot yellow leaf virus* does not require a helper virus and onward transmission in carrot crops will occur. Foliar symptoms are thought to be an upright growth habit and yellowing of foliage (see Figure 14). The virus was previously known to be transmitted by *C. aegopodii*, the willow-carrot aphid, *C. pastinaceae*, the parsnip aphid, and *C. theobaldi*, the willow-parsnip aphid. Transmission work carried out during this study has also demonstrated the ability of *Myzus persicae*, the peach-potato aphid, to transmit the virus. This study has shown the virus to be present in a wide range of apiaceous weed hosts as well as carrot crops, however, the relative importance of each virus source is not yet known.

Figure 14. Yellowing of foliage caused by viral infection in carrot. As this carrot contained multiple viruses the symptom cannot be definitively linked to infection with CYLV.

Carrot clostero virus-1 (CtCV-1)

This virus was first described through sequencing findings during FV 382a. Genetically the virus is very similar to CYLV, and is assumed to have a similar biology. Vectors, modes of transmission and field symptoms have not yet been confirmed for this virus. The findings of this study are the first step in showing this virus is widespread in weeds and carrots both in the UK and further afield. Further biological characterisation work is ongoing.

Carrot torrado virus (CaTV)

This virus was also first described through sequencing findings during FV 382a. This virus belongs to a recently discovered genus, the torradoviruses (van der Vlugt, 2015). Most members of this genus are tomato affecting viruses, and CaTV is the first virus in this group to affect the *Apiaceae*. The tomato infecting torradoviruses are known to be whitefly transmitted. The results of this study represent the first demonstration of aphid transmission of a torradovirus, with the virus being shown to be transmissible by both *C. aegopodii*, the willow-carrot aphid, and *M. persicae*, the peach-potato aphid. The virus is not currently thought to cause an observable symptom, but may contribute to yield reduction. As the virus was not

detected from any of the weeds tested in this study it is likely that infected carrots are the source of carrot epidemics. Seeds were shown to be contaminated with the virus, but further work is needed to demonstrate the importance of seed-borne infections.

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