Project title:	**Investigating the timing of transmission of carrot viruses to improve management strategies**
Project number:	FV 460
Project leader:	Dr Adrian Fox, Fera Science Ltd
Report:	Yr 2 Annual report, March 2022
Previous report:	na
Key staff:	Prof. Rosemary Collier, University of Warwick
Location of project:	York and Warwick
Industry Representative:	Ian Holmes, Strawsons Howard Hinds, Root Crop Ltd
Date project commenced:	January, 2019

DISCLAIMER

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.

Dr Adrian Fox

Senior Plant Virologist

Fera Science Ltd

Signature .... Date ....

Prof. Rosemary Collier

Warwick Crop Centre

University of Warwick

Signature .... Date ....

Report authorised by:

[Name]

[Position]

[Organisation]

Signature .... Date ....

[Name]

[Position]

[Organisation]

Signature .... Date ....

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GROWER SUMMARY

Headline

Following the discovery of several novel viruses infecting carrots in the UK, research is investigating the key vectors and timing of transmission of carrot yellow leaf virus and carrot red leaf virus. Flights of Willow-Carrot Aphid appear to track well with transmission of carrot red leaf virus.

Background

Within carrot crops the key viruses of concern are carrot necrotic dieback virus; Carrot yellow leaf virus and the viruses of the carrot motley dwarf complex, the principal virus of which is Carrot red leaf virus (CtRLV). Carrot necrotic dieback virus (CNDBV, formerly Anthriscus strain of *Parsnip yellow fleck virus*) and carrot yellow leaf virus (CYLV) are also viruses which can have a major impact on carrot crops. Previous work (FV 382 a and b) indicated that CNDBV is not a major disease observed in mature carrot crops. This may be the consequence of the virus being associated with seedling death, reducing the incidence of the virus from previous field samples. However, these previous studies indicated that both CtRLV and CYLV can be present at very high incidences (up to 100% of sampled plants). CtRLV is a persistently transmitted virus and facilitates the transmission of two other pathogenic viral agents (carrot mottle virus and carrot red leaf associated viral RNA) of the Carrot Motley Dwarf complex (CMD). CMD is associated with leaf reddening and mottling. There are no available data on yield losses associated with CMD but the complex has been linked to an impact on marketable yield through excessive lateral root hair development and root splitting (kippering). CYLV was the subject of previous AHDB funded studies (FV 382 a and b). Whilst there are no available data on yield losses associated with this virus, the previous studies strongly implicated this virus with quality losses due to development of internal necrosis in carrot root (Adams et al. 2014). Therefore, this study focuses on CtRLV as a proxy for transmission of the CMD virus complex, and CYLV as a virus thought to be present in high incidence for which minimal epidemiological information is available.

The aim of this study is to identify the timing of transmission of CtRLV and CYLV throughout the growing season and to correlate this to aphid flight data gathered from yellow water pan traps in the field. A further objective of the project is to compare the different methods used for monitoring aphid flights (Suction trapping and in-field yellow water traps), and also to see whether these new data can be used to refine the current models used for predicting flights of willow-carrot aphid (*Cavariella aegopodii*).

Summary

Year 1 (2019)

Greater virus transmission was recorded in the trials at Warwick than at Stamford Bridge. Most of the virus detected throughout the growing season was carrot red leaf virus (CtRLV) at both sites, with carrot yellow leaf virus (CYLV) being occasionally detected throughout the season. Aphid flights at both sites followed a similar pattern throughout the season, though fewer aphids were caught in the traps at Stamford Bridge. At Stamford Bridge CYLV was detected in a single week, from one bulk sample (Week of 21-May). Peak transmission at the Yorkshire site was just under 4.5% transmission, in the week of the 14 May. The trials at Stamford Bridge did not show a good relationship between aphid flights and virus, a reflection of the limited virus transmission at the Stamford Bridge site.

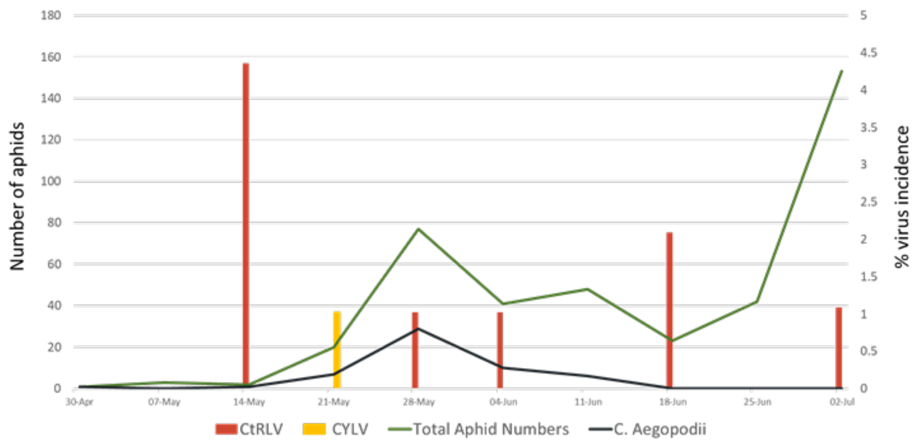

Figure1. Showing the limited virus transmission recorded at Stamford Bridge, Yorkshire. Virus content in plots is shown in the bars (Red for CtRLV, yellow for CYLV), and aphid flight data in the lines on the graph (Green for total aphid flights, Black for willow-carrot aphid).

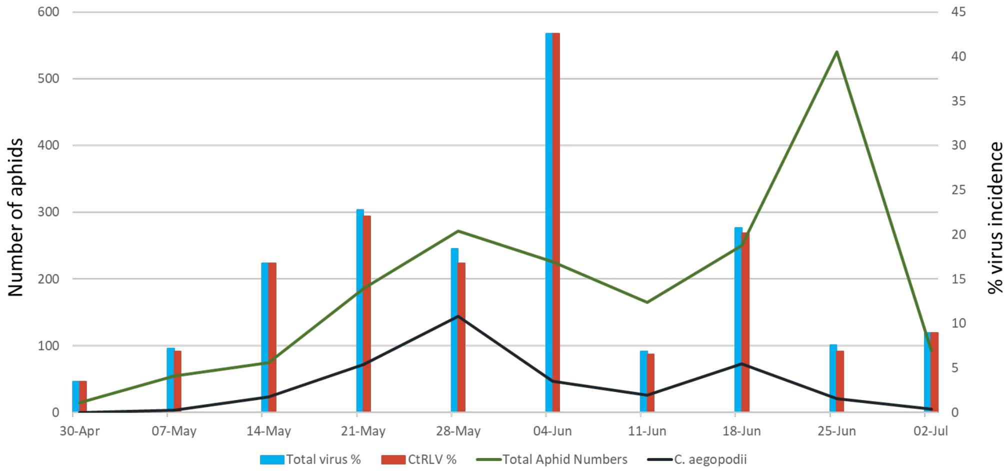

Figure 2. Virus transmission recorded in trial plots at Warwick University. Virus content is shown in the bars (Blue for total virus content, Red for CtRLV), and aphid flights in the lines on the chart (Green for total aphids, Black for willow-carrot aphid)

The trials at Warwick had greater incidence of virus transmission throughout the season, with a peak transmission of 43% in the week 4-June. Carrot yellow leaf virus was only detected sporadically throughout the season, in the weeks 7-May, 21-May, 28-May*, 11-June, 18-June, 25-June*. To reduce diagnostic costs all samples were tested as “pooled leaves”, also termed “bulk samples”. Each week 100 leaves from the test plot were sampled as 25 4-plant bulks. The percentage of virus incidence was then calculated based on the number of bulks testing positive each week. All findings were a single positive bulk per week, except * where there were two positive bulks detected. From looking at the pattern of flights of the individual aphid species at Warwick, transmission appears to track movements of *Cavariella aegopodii,* but this will be further refined in the coming seasons.

Year 2 (2021)

Following a year hiatus due to COVID affecting the ability of staff at both Warwick and Fera to conduct field work, the year 2 of the trial was rolled over to 2021. The trial at Fera was conducted at a field in Buttercrambe, less than 2 km North of the Stamford Bridge site used in 2019. The first week of the trial (uncover and aphid trapping) was approximately 2-3 weeks later than in 2019, occurring in the week of 18 May, rather than 30th April, however, this aligned well with the relative aphid predictions and the relative abundance of aphids caught at both the Fera and Warwick site were in line with a similar the phenology (timing of the life cycle) of the various species across both years of the trial.

In a similar pattern to the 2019 trial, there was very little transmission recorded in the Fera trial, with a maximum transmission of 1% of any virus across the entire trial in the weeks of

15 June, 6 and 13 July. Aphid numbers were negligible throughout the season. C. aegopodii remained low throughout the entire season rarely getting above single figures in any week. Consequently, with both transmission and vector numbers so low, it is difficult to draw any further conclusions from this part of the trial.

The pattern of virus transmission and aphid catches on the Warwick trial are shown in figure 3. Transmission increased rapidly in the early weeks of the trial (18 May – 8 June), peaking on 1 June, where all plant samples tested were positive for virus, with 95% of the virus detected being CtRLV. Carrot yellow leaf virus was also detected in the weeks of 1 and 8 June, although this was only present at low incidence (~5% of virus detected). Throughout this early part of the trial vector numbers corresponded well to transmission, with the majority of aphids caught in yellow traps being the willow carrot aphid. Later in the trial (29 June onwards) a second peak of virus transmission was recorded, which do not correspond with a rise in numbers of willow-carrot aphid. However, during this period there was a rise in the catches of *C. pastinaceae* (parsnip aphid) representing a large proportion of the small peak in aphid catches at 6 July. It should be considered that this species, not identified as a factor in the previous trial, may be driving this late season transmission.

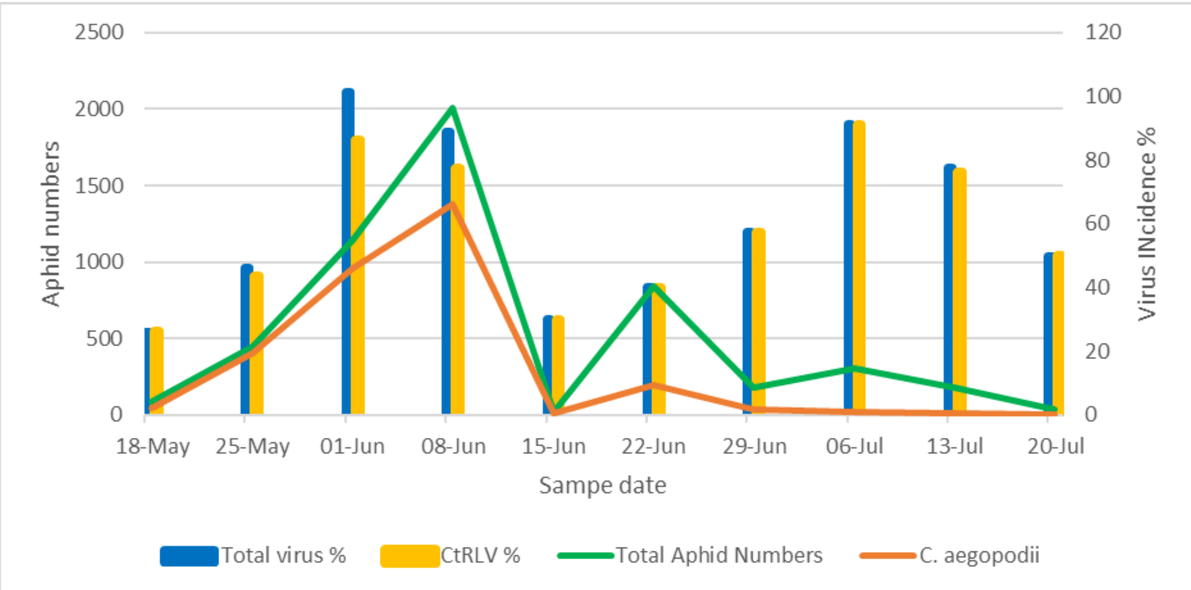

Figure 3. Virus transmission recorded in trial plots at Warwick University. Virus content is shown in the bars (Blue for total virus content, Yellow for CtRLV), and aphid flights in the lines on the chart (Green for total aphids, Orange for willow-carrot aphid)

Comparisons of monitoring data collected in different ways (plant sampling, suction traps, water traps) suggest that all approaches are broadly measuring the ‘same thing’. Additionally, on the strength of these data the day-degree forecast for willow-carrot aphid (*C. aegopodii*) appears to be relatively robust, whereas it may be more difficult to forecast the activity of peach-potato aphid (*M. persicae*) and the parsnip aphid (*C. pastinaceae*).

Financial Benefits

As this report is based on trials to assess the timing of transmission of viruses to inform a subsequent control trial, financial benefits for growers cannot be assessed at this point. In year 3 of the project (2022) the focus of field work will be on a control trial to look at optimising control strategies through a replicated block trial based at Warwick crop centre.

Action Points

As these are first year data of a multi-year project, there are no action points for growers at this stage.

SCIENCE SECTION

Introduction

The initial steps of assessing presence, incidence and impact are essential to being able to apply appropriate control measures. The epidemiology of a vector borne virus depends upon a few key factors such as the main vector species driving epidemics, the sources of viruses infecting crops, and consequently the timing of transmission and these data can be used to formulate an effective control strategy. This 'formula' for aphid-vector-host interactions can also be exploited to allow inferences to be made regarding data gaps, for instance by correlating the timing of transmission with aphid flight data, inferences can be made regarding the key vector species driving transmission. Within carrot crops the key viruses of concern are carrot necrotic dieback virus (CNDBV, formerly Anthriscus strain of *Parsnip yellow fleck virus*), carrot red leaf virus (CtRLV) and carrot yellow leaf virus (CYLV). Previous work (FV 382 a and b) indicated that CNDBV is not a major disease observed in mature carrot crops. This may be the consequence of the virus being associated with seedling death, reducing the incidence of the virus from previous field samples. However, these previous studies indicated that both CtRLV and CYLV can be present at very high incidences (up to 100% of sampled plants). CtRLV is a persistently transmitted virus and facilitates the transmission of two other pathogenic viral agents (carrot mottle virus and carrot red leaf associated viral RNA) of the Carrot Motley Dwarf complex (CMD). CMD is associated with leaf reddening and mottling. There are no available data on yield losses associated with CMD but the complex has been linked to an impact on marketable yield through excessive lateral root hair development and root splitting (kippering). CYLV was the subject of previous AHDB funded studies (FV 382 a and b). Whilst there are no available data on yield losses associated with this virus, the previous studies strongly implicated this virus with quality losses due to development of internal necrosis in carrot root (Adams et al. 2014). Therefore, this study focuses on CtRLV as a proxy for transmission of the CMD virus complex, and CYLV as a virus present in high incidence for which minimal epidemiological information is available.

Even within aphid-transmitted viruses there are a range of transmission mechanisms which determine the time taken to acquire and pass on a virus and the range of aphid vectors able to transmit each virus. Non-persistently and semi-persistently transmitted viruses (e.g. Carrot yellow leaf virus, CYLV) are rapidly acquired and transmitted (less than a few minutes and through probing behaviour). The consequence of this is that chemical control measures without a rapid knockdown effect may only have a limited effect on transmission. The persistently transmitted viruses, such as Carrot red leaf virus (CtRLV) have a closely evolved relationship with their aphid vector, requiring the presence of a bacterial symbiont for circulation through the aphid body. This tight relationship means that these viruses tend to be transmitted by a more limited range of vector species and transmission can take longer (at least hours) to occur. Through laboratory studies, multiple vector species may be implicated in the transmission of viruses (Naseem et al. 2016; Rozado-Aguirre et al. 2016). These studies may indicate the relative efficiency of different species, for instance previous work in potato (Lacomme et al. 2017; Fox et al. 2017a), however, this potential to transmit a virus may not directly correlate with the field epidemiology of a virus with more numerous but less efficient vectors (Lacomme et al. 2017). By examining when each vector species is migrating into a crop and correlating these data with the timing of transmission of key viruses researchers can identify both the species most closely associated with the transmission of viruses and give supporting data on the optimum time for control measures to be applied.

The key aphid species associated with transmission of CtRLV and CYLV are *Cavariella aegopodii* and *Myzus persicae* (Naseem et al. 2016; Rozado-Aguirre et al. 2016; Elnagar and Murant 1978). The AHDB-funded projects SCEPTRE (Horticulture LINK), SCEPTREplus and FV 445 have investigated control of *C. aegopodii* and *M. persicae* (SCEPTREplus only) with insecticides and biopesticides and this research will be used to inform management strategies in the proposed project. The SCEPTREplus work includes a component on the persistence of treatments, which may provide additional useful information in formulating strategies. FV 445 provided proof of concept of using virus tests to evaluate the efficacy of control programmes. FV 445 also showed that it should be possible to use the yield and quality assessments of carrot roots to assess the efficacy of control programmes.

The precise timing of colonisation of crops by aphids varies from place to place and year to year and this is greatly influenced by weather conditions, particularly temperature. Potentially, as Figure 1 illustrates, there could be pressure from virus vectors (*M. persicae, C. aegopodii*) for almost 3 months. This is a long period over which to provide effective control. Thus it is important to make best use of all the information that is available on aphid phenology – both monitoring and forecasting information. At present, a basic day-degree forecast of first flight for *C. aegopodii* developed at University of Warwick is used in the AHDB Pest Bulletin. Rothamsted Research issues a forecast of the first flight of *Myzus persicae* in early March each year which is reported in AHDB Aphid News and was reported in the AHDB Pest Bulletin. Real-time information on the numbers of aphids captured in the Rothamsted suction traps is available each year (Rothamsted Insect Survey web site, AHDB Aphid News, Warwick Crop Centre Pest Blog), although obviously there is a 'delay' due to the time needed for identification of samples. Fera Science Ltd offers a monitoring service for growers using yellow water traps.

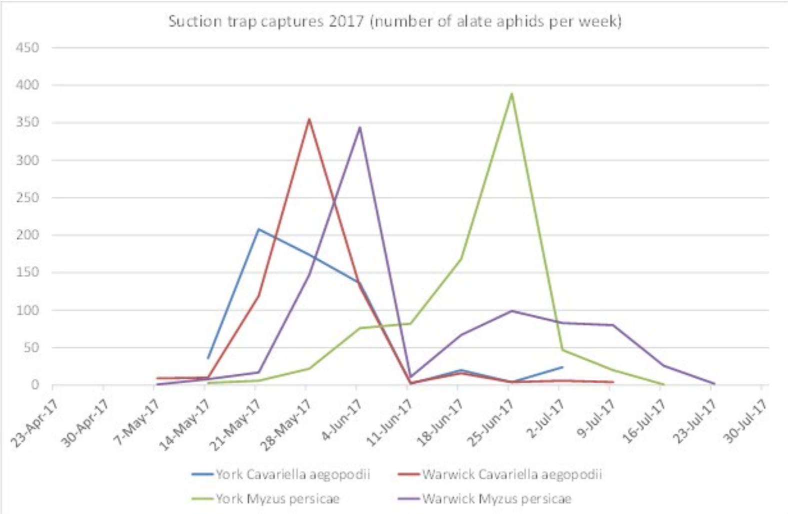

Figure 1. Numbers of alate aphids captured per week in suction traps at Fera (York) and Warwick Crop Centre (Wellesbourne, Warwick) in 2017 (data provided by the Rothamsted Insect Survey).

An additional element of the epidemiology which should be considered within an Integrated Pest Management approach are the sources of viruses, as this can inform potential cultural control approaches. Previous work carried out at Warwick Crop Centre (Defra project IF0188), examining the population genetics of CtRLV, indicated that CtRLV recovered from carrot crops was more closely related to the virus from carrot sources (wild carrots and other carrot crops) than it was to CtRLV recovered from apiaceous weed sources such as cow parsley (*Anthriscus sylvestris*). This is a strong indication that the source of CtRLV infections are originating in other carrots/carrot crops. During FV 432b these same samples were tested for the presence of CYLV and other recently discovered carrot viruses. The presence of the virus was detected in samples of cow parsley, hogweed (*Heracleum sphondylium*) and other apiaceous weeds; unfortunately, the nucleic acids in them had degraded over time and a population study on the viruses could not be completed. A further element of this current study will be to look at the presence of carrot infecting viruses in weed hosts, to try to ascertain the sources of carrot virus epidemics.

Objective 1. Vector management – assessing the relative importance of different vector species and the timing of transmission of the key viruses into carrot crops

Year 1 - 2019

Materials and methods

Timing of transmission and correlation with vector aphids

Plots in carrot fields were covered with fine mesh netting and sequentially exposed to virus vectors so that peak transmission periods can be related to the aphid species migrating into crops each week. At weekly intervals throughout the growing season a section of netting c.5 m long was rolled back on each of the 'uncovered' plots to expose the carrot crop to potential virus infection. Yellow water traps (YWT), of the design used in the AHDB aphid monitoring scheme were placed close to the exposed sections. Two (2) sites used for netting trials were situated in the 'North', within a working distance of Fera and one in the 'Midlands' at Warwick Crop Centre (Wellesbourne, Warwick) (Years 1-2). Trials were set up in the week beginning 23 April 2019 and ran through to Week beginning 1 July 2019.

Week		Week		Week
7		9		3
5		4		6
2		Uncovered Control		10
8		1		Covered Control

Figure 1.1. Plot map of the field plot at Stamford Bridge, North Yorkshire.

The plots at Fera were in a commercial carrot crop near Stamford Bridge, North Yorkshire. The trial was set out as a single randomised block trial. (See Figure 1.1) with ten (10) covering treatments and two (2) control plots (one covered all the time and one uncovered all the time).

The trial at Wellesbourne, Warwick was located in the field known as Long Meadow West and consisted of 12 beds x 23 m of drilled carrot. The seed was drilled at 100 seeds per metre with 4 rows (35 cm spacing) per bed on 22 March 2019. The trial was divided into 5 m plots with 1 m between plots (Figure 1.2) and each plot was covered with 0.6 mm insect-proof netting (Figure 1.3). Four replicate plots were sequentially exposed to virus vectors so that peak transmission periods could be related to the aphid species migrating into crops each week. There were 10 uncovering treatments plus two controls (one permanently covered and one permanently uncovered). At intervals throughout the growing season a section of netting was rolled back to expose the carrot crop to potential virus infection. The first set of plots were uncovered on 23 April 2019. Yellow water traps (YWT) were placed within the exposed sections. At the end of each week the exposed sections were re-covered and further sections exposed. The YWT were emptied and re-set next to each newly exposed section of crop. The contents of the traps were sent to Fera where the aphids were identified and counted.

Figure 1.2. Plan of uncovering trial at Wellesbourne, Warwick.

11	2	5	12	6	1	7	9	4	8	3	10	
1	2	3	4	5	6	7	8	9	10	11	12	N
8	12	4	7	3	11	10	6	2	1	5	9	↑
13	14	15	16	17	18	19	20	21	22	23	24	
1	7	10	9	2	8	12	5	3	11	6	4	
25	26	27	28	29	30	31	32	33	34	35	36	
6	3	9	5	4	10	1	11	8	12	7	2	5m
37	38	39	40	41	42	43	44	45	46	47	48	

Treatment Number	
1	Uncovered permenantly
2	Covered permenantly
3	Uncovered 23rd April
4	Uncovered 30th April
5	Uncovered 7th May
6	Uncovered 14th May
7	Uncovered 21st May
8	Uncovered 28th May
9	Uncovered 4th June
10	Uncovered 11th June
11	Uncovered 18th June
12	Uncovered 25th June

Figure 1.3. Photograph of trial at Wellesbourne, Warwick.

At the end of each week the exposed sections were re-covered, and a further section exposed in accordance with the relevant plot maps. The yellow water traps were emptied and re-set next to each newly exposed section of crop. After 4 weeks of being re-covered, 100 carrot plants were sampled from each plot and tested for the presence of CYLV and CtRLV using previously described methods from FV432 a and b and Adams et al. (2014) . Plant RNA extractions testing positive for the presence of CYLV were retained for possible inclusion in a phylogenetic study on sources of virus. The aphids present in the YWT were identified and enumerated. Relative aphid abundance in both YWT and suction trap samples was then related to the periods in which peaks of transmission occurred. Samples from covered and uncovered control plots were also taken from each trial at the end of the growing season and tested following the procedures outlined in section 2.

Year 2 - 2021

Due to the COVID pandemic, no trials were conducted during 2020 and the project was delayed a year with the second field trial year being conducted in 2021. The general approach to the trials at both York and Warwick was consistent with those conducted in 2019. Due to seasonal differences, planting and predicted first flights were approximately three weeks later than in the 2019 trial Consequently trial initiation with the first week of uncovering was the period of 11-18 May 2021, with the first week of field sampling taking place in the week of 8th June (week 1 uncovered plants). The final week of uncovering fell on the week of the 13 July 2021, with weekly plot sampling continuing until the week of 10th August (week 10 uncovered plants). Sampling and testing of plant samples and aphid catches were as described in 2019.

There were changes to the plot layout due to the randomised plot nature of the trial as shown in figure 1.3.1 example below representing the 2021 “Fera” trial at Buttercrambe, York.

Week	Week	Week
9	8	4
COVERED	3	7
2	6	UNCOVERED
5	10	1

Figure 1.3.1 Plot plan for the 2021 Fera (Buttercrambe, York) site

Results

Year 1 - 2019 trials

Captures of aphids in yellow water traps in the trial plots

Aphid captures at Wellesbourne, Warwick are shown in Figure 1.4. Of the aphids that are known to infest carrot, willow-carrot aphid (*C. aegopodii*) was the most abundant.

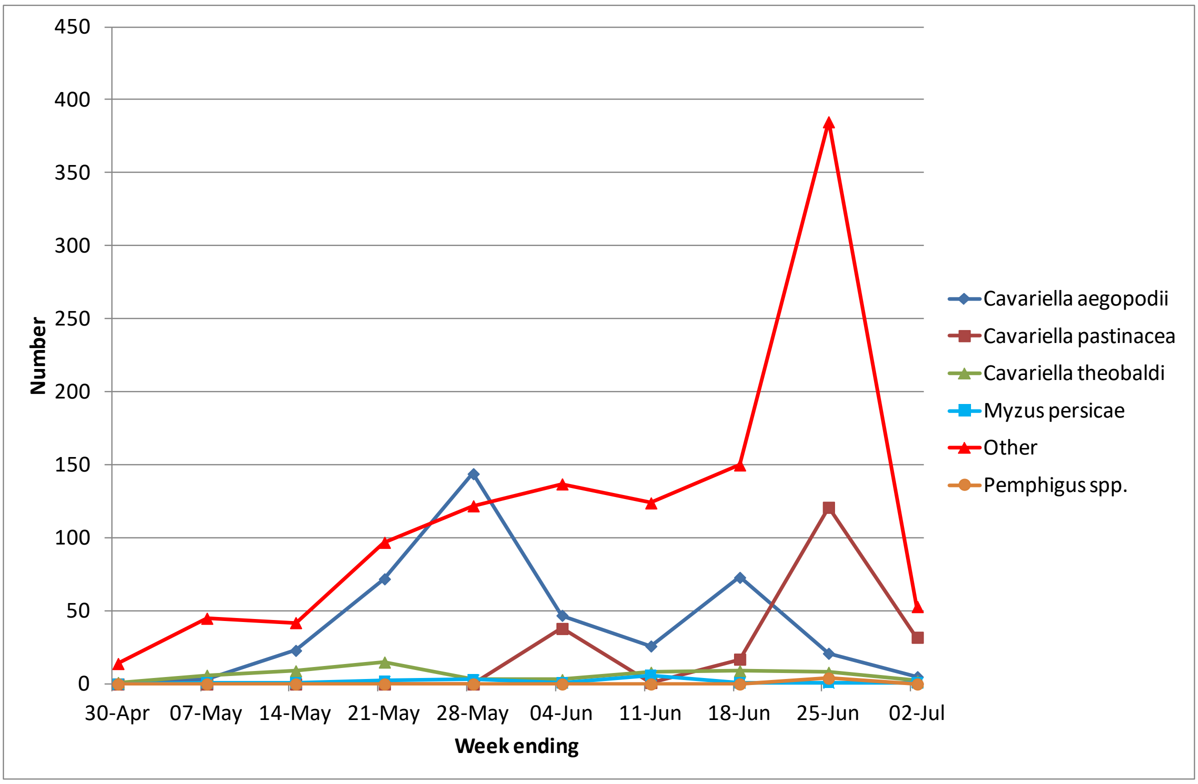

Figure 1.4 Total numbers of aphids captured per week in 4 water traps at Wellesbourne, Warwick in 2019. *C. pastinacea* and *C. theobaldii* are parsnip aphids.

Timing of transmission and correlation with vector aphids

Plots were laid out in the accordance with the plot maps. The results of total transmission (% virus incidence per week) and aphid numbers in the plot traps are presented in Figure 1.5 (Stamford Bridge data) and Figure 1.6 (Warwick data). There was little virus transmission recorded in the crop at Stamford Bridge, with a maximum weekly transmission under 4.5% virus. This occurred in a week with virtually no aphid activity recorded (a solitary *C. aegopodii*) These low levels of transmission are supported by anecdotal reports relating to the field as a whole having very low virus incidence. Due to these low levels of transmission there is little further analysis can be carried out from these data.

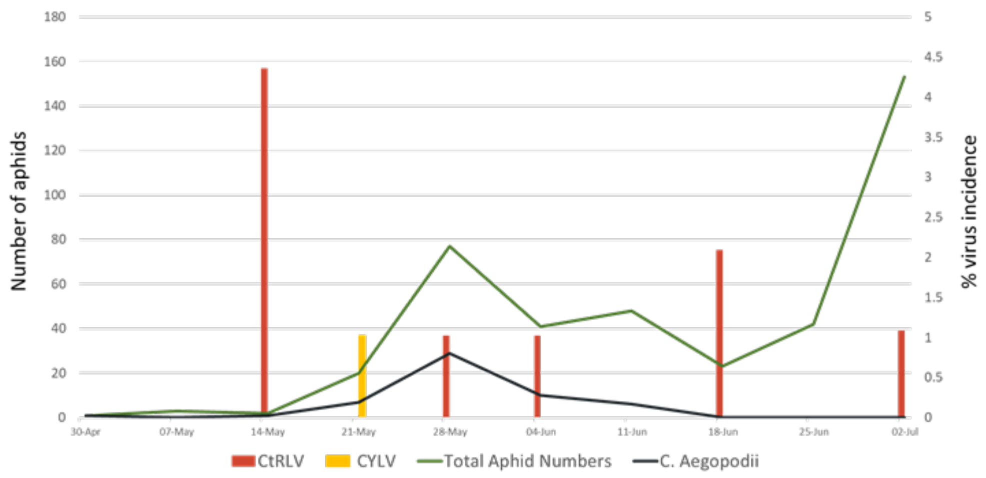

Figure 1.5. Weekly virus incidence and aphid numbers (Yellow water trap data) at Stamford Bridge, North Yorkshire. Virus data presented as % of individual viruses due to low incidence of virus transmission.

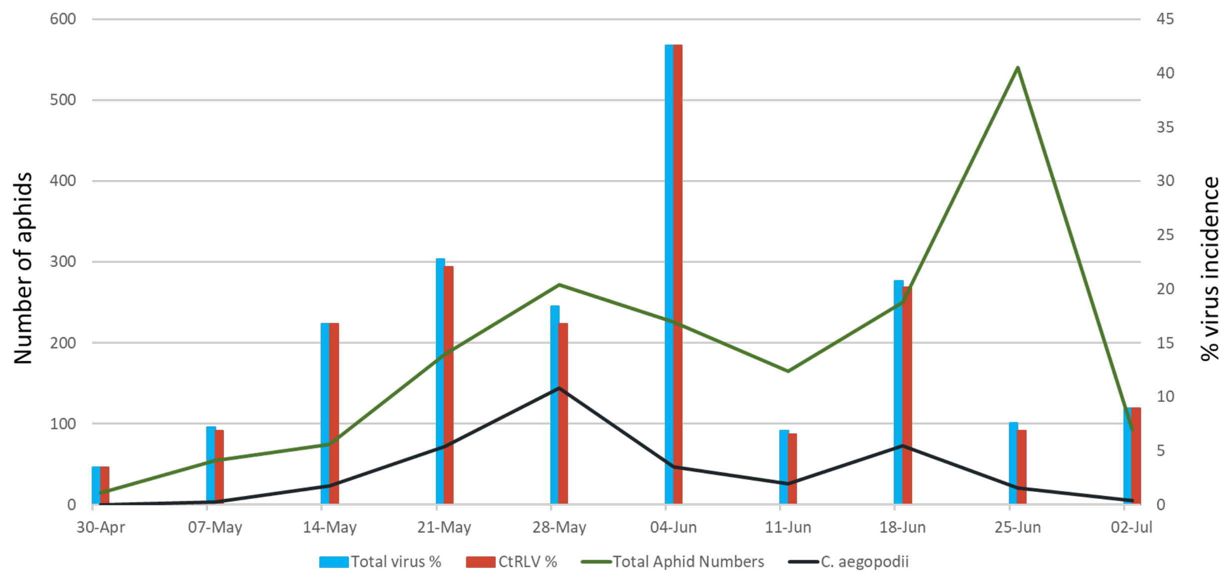

Figure 1.6. Weekly virus incidence and aphid numbers (yellow water trap data) at University of Warwick, Warwickshire. Virus data presented as % total virus and % carrot red leaf virus. Results are presented collated across all replicated plots.

Transmission in the Warwick trial (Figure 1.6) was at a much higher rate than at Stamford Bridge throughout the entire season. Week to week virus incidence rose steadily through the first four weeks of the trial and after a slight drop in incidence in week 5 (28 May) recorded a peak transmission rate of just under 43% (4 June). The majority of virus transmission detected was CtRLV, with only occasional sporadic transmission of CYLV throughout the season.

It should be noted that aphid numbers from Warwick represent catches from four traps, whereas numbers from Stamford Bridge are the total from two traps. Even accounting for twice as many traps at Warwick, the relative numbers of aphids caught were higher at Warwick than at Stamford Bridge. Aphid numbers caught in yellow water traps at both sites showed a similar weekly increase through the early weeks of the trial (Figures 1.5 and 1.6). With a peak of *C. aegopodii* (willow carrot aphid) in week 5 (28 May). Aphid numbers reduced mid-season and went on to peak in week 9 (25 June) at Warwick and a week later at Stamford Bridge. This late peak at Stamford Bridge was due to a late migration of *Cavariella pastinacea* (parsnip aphid). Although there was a large number of *C. pastinacea* in the trap during the peak in week 9, there was also a large number of ‘other’ species present.

2019 Yield data

The plots at Wellesbourne were also assessed for yield and quality by lifting a fixed length of row from each plot (1.3m x 4 rows) on 19-20 November 2019, washing the roots and then assessing, counting and weighing them. They were also scored for damage by carrot fly larvae. Figure 1.7 shows the mean yield in kg per plot. Figure 1.8 shows the percentage of carrot roots not damaged by carrot fly on 19-20 November. Levels of carrot fly damage were very high, suggesting some movement of carrot fly larvae under the covers, and confounding the assessment of the impact of virus load on the yield of carrots.

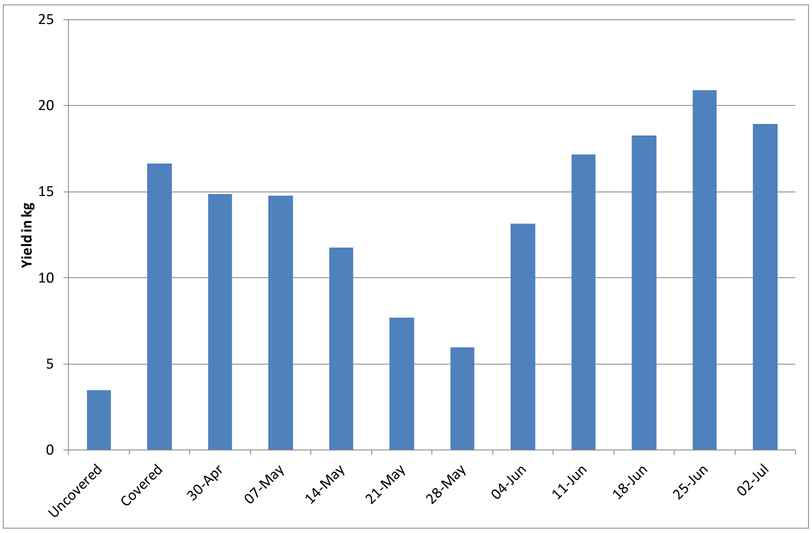

Figure 1.7 Yield in kg of carrots per plot (sample size 1.3m x 4 rows) on 19-20 November 2019.

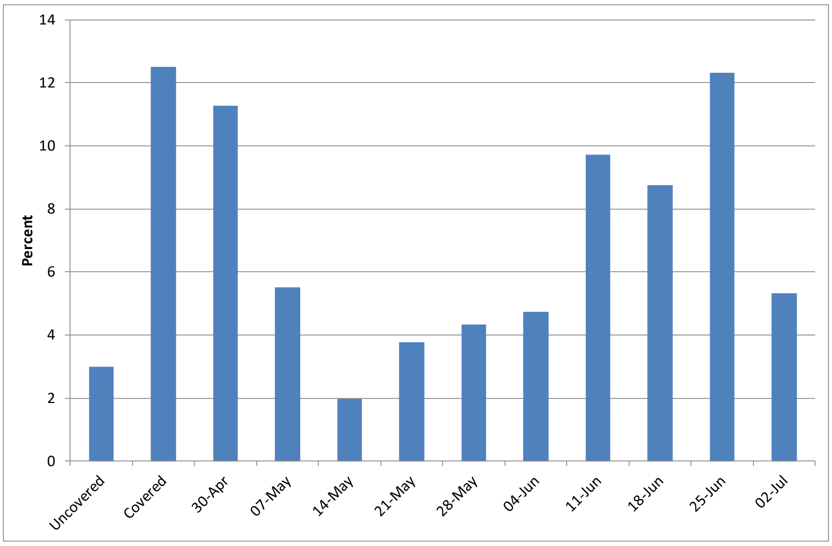

Figure 1.8 Mean percentage carrot roots undamaged by carrot fly (less than 5% of root surface damaged) (sample size 100 roots) rows) on 19-20 November 2019.

Year 2: 2021 trials

Captures of aphids in yellow water traps in the trial plots

Numbers of aphids caught in the traps in the Warwick trial (figure 1.9) were markedly higher in 2021 than in 2019 (figure 1.4). *Cavariella aegopodii* (willow-carrot aphid) was the most numerous species present. A difference to the pattern of aphid movements relative to that observed in 2019 was the much earlier flight of *C. aegopodii*. The later flight of *C. aegopodii* in 2019 masked a subsequent smaller migration of *C. pastinaceae*.

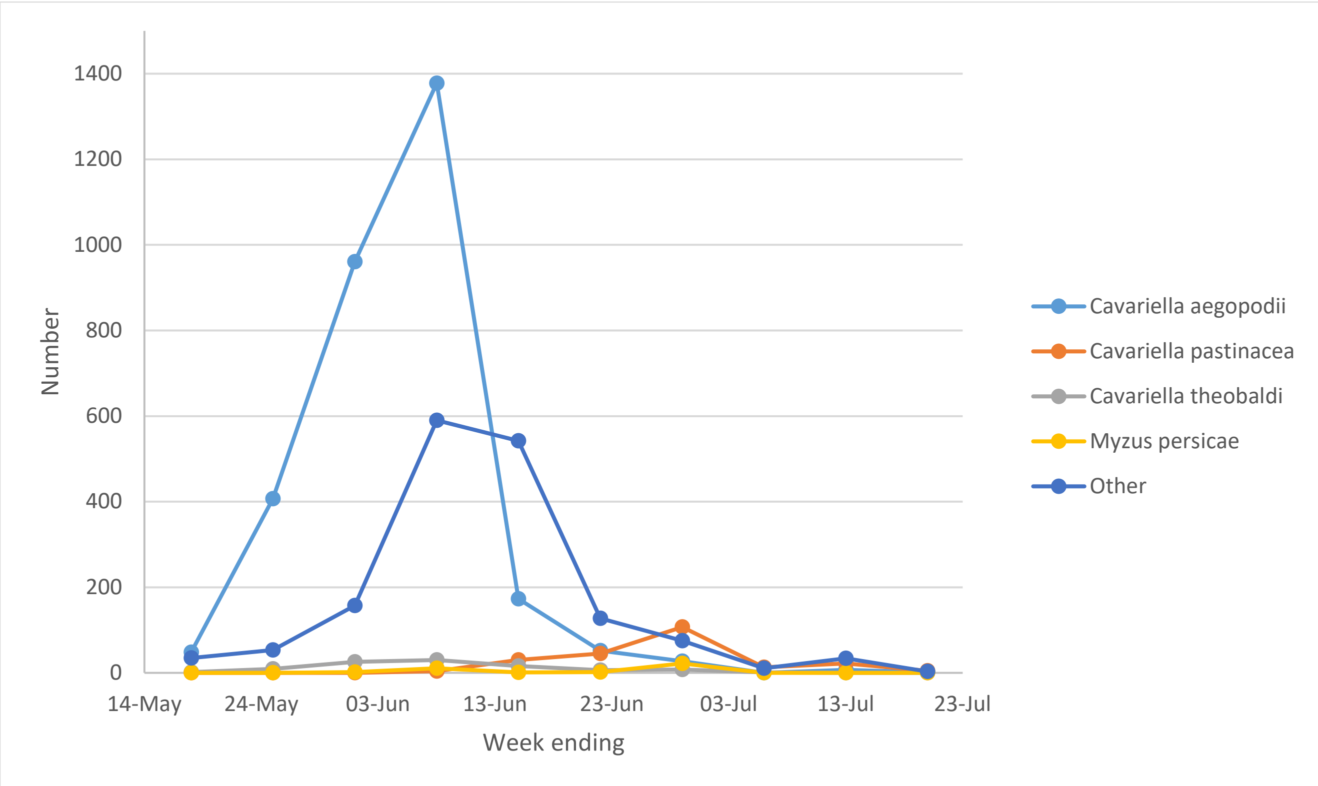

Figure 1.9 Timing of aphid catches in Warwick trial, all traps combined

Timing of transmission and correlation with vector aphids

The results of total transmission (% virus incidence per week) and aphid numbers in the plot traps are presented in Figure 1.10 (Fera-Buttercrambe data) and Figure 1.11 (Warwick data). At the Fera trial there was little virus transmission recorded for the second year, with a maximum weekly transmission of 1% virus (calculated mean incidence). This occurred in a week with virtually no aphid activity recorded and again similar levels were recorded on a week with relative high activity but little *C. aegopodii*. Due to these low levels of transmission, there is little further analysis can be carried out from these data, and consequently no comparative data with 2019 and 2021 are presented.

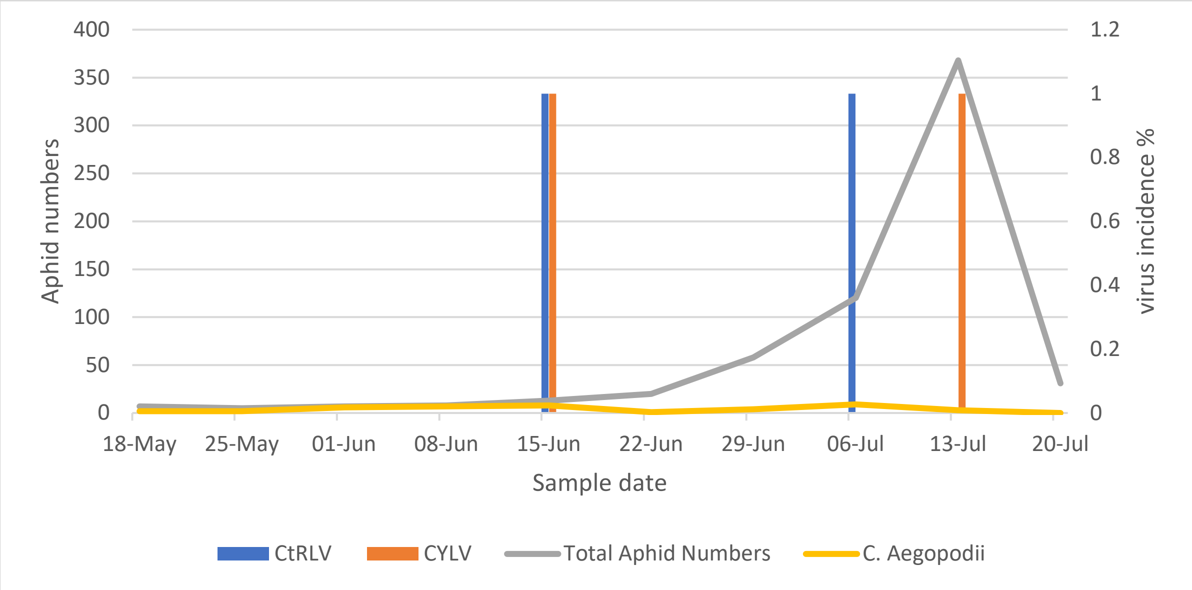

Figure 1.10 Incidence of carrot red leaf virus and carrot yellow leaf virus and aphid catches in the 2021 Fera-Buttercrambe trial

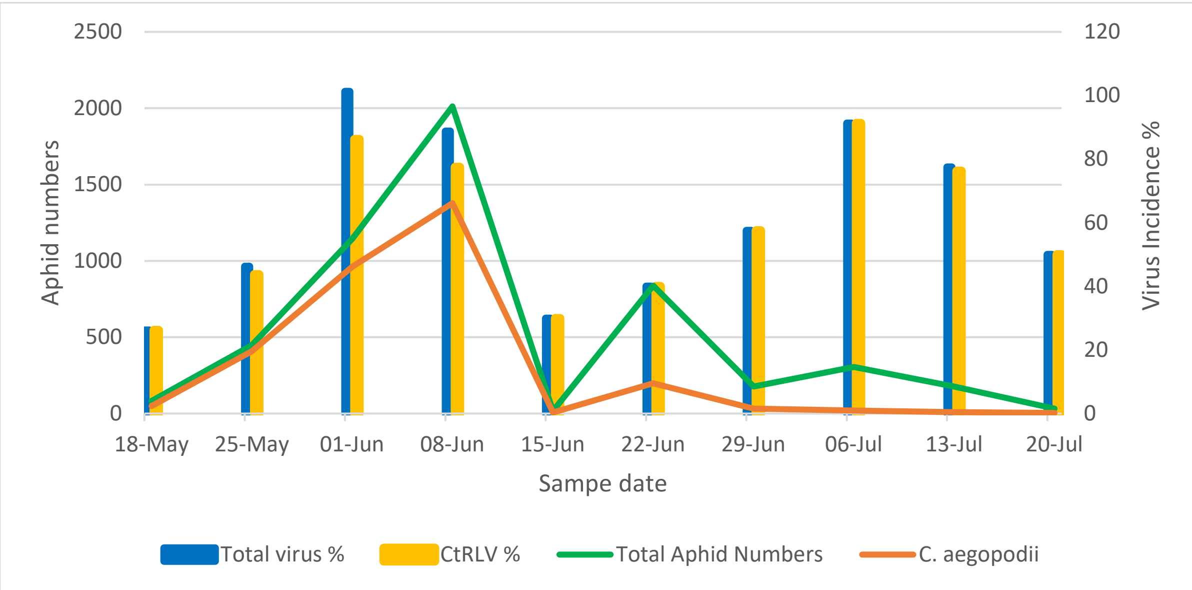

Figure 1.11 Incidence carrot red leaf virus and carrot yellow leaf virus and aphid catches in the 2021 Warwick trial

Aphid catches and recorded virus transmission at the Warwick trial site are shown in Figure 1.11. Throughout the trial the main virus transmitted was carrot red leaf virus, being the only virus recorded in six of the ten weeks of the trial. The highest incidences of CtRLV were recorded in the weeks of 1 June (91.25% incidence) and 6 July (93.75% incidence). CYLV was recorded in four weeks, namely, 25 May (2.5% incidence) 1 June (15% incidence), 8 June (11.25% incidence) and 13 July (1.25%). Early in the season (up to week of 15 June) the largest relative number of aphids was *C aegopodii* and it is likely that this species is driving early season transmission. The later season peak in CtRLV transmission does not coincide with migrations of *C. aegopodii*. However, a large proportion of the small peak in aphid numbers around early July (29 June through 13 July) is represented by *C. pastinaceae* (parsnip aphid) (Figure 1.9 and 1.11) and it is likely that this species may play a role in late season transmission of the viruses in this study.

Objective 2. Sources of virus

Materials and Methods

1. Samples of apiaceous weeds from around carrot fields were collected and tested for the presence of target viruses
   1.1. Samples from each trial field were collected
   1.2. Samples were collected at two (2) time points approximately 6 weeks apart, in May and June.
   1.3. RNA was extracted and tested for the presence of CtRLV and CYLV from samples in accordance with previously described methods from FV432 a and b and Adams et al. (2014)

Results

112 samples of weeds were collected from fields at Warwick and Stamford Bridge during 2019. RNA was extracted from these samples and initial testing has been carried out to check extraction quality. Testing indicated a higher incidence of CtRLV in cow parsley (*Anthriscus sylvestris*) (Table 2.1) CYLV was not detected from any of the hogweed samples however it was detected from five cow parsley plants sampled at the Fera - Stamford Bridge site.

Table 2.1 Results from testing apiaceous weed from

	Warwick			Fera		
	Samples	CtRLV	CYLV	Samples	CtRLV	CYLV
Hogweed	46	0	0	10	1	0
Cow parsley	46	9	0	10	8	5

Weed samples were not drawn during the 2021 trials. Due to the low sample numbers positive, it is unlikely that meaningful inferences can be drawn on the origins of the viruses detected in the trial and the capacity from this sampling effort was rolled forwards into supporting the final year trial on control options. To further investigate the role of wild hosts in the carrot virus pathosystem work is being conducted under the Euphresco project “Baseline surveillance for virus reservoirs” using samples gathered from the BBSRC Bacterial Plant Diseases project “CALIBER”. The extent of sampling and sequencing within these projects should allow enough data to be generated to build viral networks (e.g. Figure 2.1) to better understand the host relationships of genotypes of these viruses. These data are being combined with previously generated data (e.g. Defra IF0118) to give greater depth of information on the associations between viruses and their hosts based on sequence level data, which should indicate if there are host specialisations, information gathered from these other projects will be included within the final report for this project.

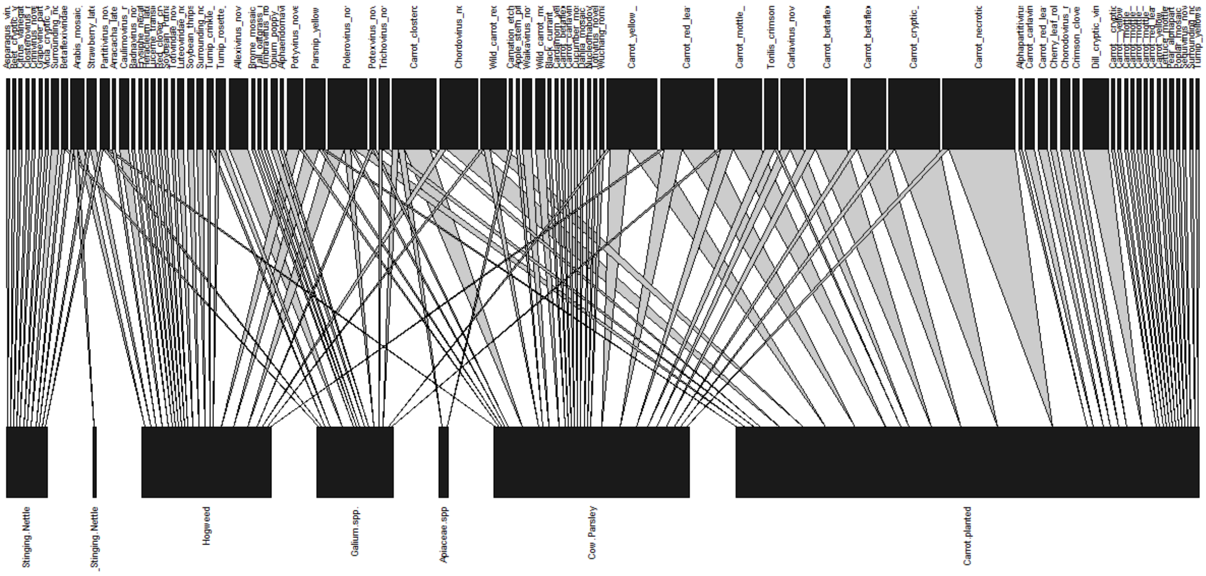

Figure 2.1 Network diagram composed of virus sequence data generated from carrot fields and associated weed samples, showing associations between known and novel viruses and the hosts in which they were detected.

Objective 3. Further development/refinement of aphid forecasting systems and improved interpretation of monitoring data

3.1 Comparison of methods of monitoring aphid infestations (on plants, suction trap, water trap) – field data 2019.

At Wellesbourne, plots of carrots are maintained throughout the year to support the population of carrot fly. The carrots are overwintered, usually under covers, and then uncovered. New plots of carrots are sown in late March and then in May. Numbers of aphids (primarily willow-carrot aphids) were monitored on these plots in spring 2019. Table 3.1 shows the numbers of aphids on the overwintered carrots from mid-March, Aphids were present from 21 March when sampling started and numbers of winged and wingless aphids peaked in late April, declining considerably by late May. The numbers of parasitized aphids (aphid mummies) were also recorded.

Table 3.1 Numbers of aphids on 3 x 0.5 m lengths of row of overwintered carrots at Wellesbourne in 2019.

Date	**Numbers of aphids**		
2019	**Winged**	**Wingless**	**Parasitised aphids**
21 March	1	172	0
27 March	2	288	0
5 April	5	448	3
10 April	5	535	2
18 April	51	1245	16
25 April	74	2640	11
1 May	17	226	11
9 May	4	48	18
14 May	0	1	8
22 May	0	2	7

Aphids were also monitored on the new carrots sown in late March 2019 (Table 3.2). Winged aphids had arrived in the plots by 9th May and numbers of winged aphids peaked in mid to late May. Numbers of wingless aphids peaked in early June and then declined over time, there being no aphids on the plants by early August. Numbers then started to increase again in early September. Numbers of parasitized aphids peaked in mid-June and ladybirds were also present in the plots from late May – mid June.

Table 3.2. Numbers of willow-carrot aphid on 3 x 0.5 m lengths of row of newly-sown carrots (March) at Wellesbourne in 2019 (Long Meadow Centre – LMC; Long Meadow West – LMW).

Date	Numbers of aphids and ladybird larvae			
2019	Winged	Wingless	Parasitised aphids	Ladybird larvae
25 April	LMC 0, LMW 0	LMC 0, LMW 0	LMC 0, LMW 0	
1 May	LMC 0, LMW 0	LMC 0, LMW 0	LMC 0, LMW 0	
9 May	LMC 0, LMW 2	LMC 3, LMW 8	LMC 0, LMW 0	
14 May	LMC 4, LMW 11	LMC 1, LMW 4	LMC0, LMW 0	
22 May	LMC 22, LMW 23	LMC 8, LMW 14	LMC2, LMW 1	
30 May	LMC 25, LMW 16	LMC 27, LMW 47	LMC 2, LMW 5	LMC 1, LMW 0
6 June	LMC 21, LMW 4	LMC 391, LMW 344	LMC 2, LMW 6	
13 June	LMC 3, LMW 10	LMC 133, LMW 266	LMC 2, LMW 7	LMC 3, LMW 6
20 June	LMC 2, LMW 5	LMC 162, LMW 221	LMC 2, LMW 10	
27 June	LMC 1, LMW 2	LMC 95, LMW 143	LMC 1, LMW 9	
4 July	LMC 1, LMW 2	LMC 17, LMW 18	LMC 1, LMW 1	
11 July	LMC 1, LMW 0	LMC 2, LMW 22	LMC 1; LMW 0	
17 July	LMC 0, LMW 0	LMC 5, LMW 12	LMC 0, LMW 1	
24 July	LMC 0, LMW 0	LMC 4, LMW 1	LMC 0, LMW 0	
6 August	LMC 0, LMW 0	LMC 0, LMW 0	LMC 0, LMW 0	
20 August	LMC 0, LMW 0	LMC 0, LMW 0	LMC 0, LMW 0	
4 September	LMC 1, LMW 1	LMC 21, LMW 10	LMC 0, LMW 0	
19 September	LMC 0, LMW 0	LMC 42, LMW 6	LMC 0, LMW 0	
3 October	LMC 1	LMC 38	LMC 0	

Summary data for willow-carrot aphid from the network of Rothamsted Suction traps are shown in Table 3.3.

Table 3.3. Summary of captures of willow-carrot aphid in 2019 by the network of suction traps run by Rothamsted Research and SASA (from the weekly bulletins). The trap at East Malling was not running until late in the year.

Cavariella aegopodi	Inverness	Dundee	Edinburgh	Ayr	Newcastle	FERA, York	Preston	Kirton	Broom's Barn	Wellesbourne	Hereford	Rothamsted	Writtle	Ascot	East Malling	Starcross	Total
Week ending																	**0**
24-Feb													1				**1**
03-Mar							1										**1**
10-Mar																	**0**
17-Mar																	**0**
24-Mar																	**0**
31-Mar																1	**1**
07-Apr																	**0**
14-Apr																	**0**
21-Apr		1				1	4						1			12	**19**
28-Apr			2				4	2		1			1			5	**15**
05-May			3	1			14	3	3	2	1		1	10		8	**46**
12-May		1	2			9	16	1	3	10	7	3	6	21		14	**93**
19-May	9	`4	52	2		154	140	16	15	85	64	19	10	20		22	**608**
26-May	4	60	54	1	9	206	126	100	106	244	63	106	11	68		21	**1179**
02-Jun	14	54	132	15	2	129	48	93	49	65	38	33		30		51	**753**
09-Jun	3	38	36	16	5	28	49	21	14	28	58	8	21	1		10	**336**
16-Jun		3	13	1	4	6	24	5	4	15	19	1	11			24	**130**
23-Jun		3	1	1		16	20	14	26	46	60	28	48	35		25	**323**
30-Jun	1	4	2			19	23	19	6	18	27	10	1	4		26	**160**
07-Jul			3	2		42	2	4	6	4	5	1	1	5		4	**79**
14-Jul		2	1		4	7	6	4	2	14	9	2	2				**53**
21-Jul					1			2	1	1	3		2	1		3	**14**
28-Jul	1						2	2			1					1	**7**
04-Aug	1						1			1	1						**4**
11-Aug			1														**1**
18-Aug		1														1	**2**
25-Aug		5	1			1			1								**8**
01-Sep																	**0**
08-Sep		22															**22**
15-Sep		17					1	1									**19**
22-Sep	2	30	5			6	2	1					1				**47**
29-Sep		553	10		3	16	12	5									**599**
06-Oct	7	142	51	1	1	27	4	23	29				1			2	**288**
13-Oct		298	1			15	30	105							1		**450**
20-Oct		452	6		1	102	1	86	14	1					1		**664**

Figure 3.1 compares the pattern of captures of willow-carrot aphid in the suction trap and the water traps at Wellesbourne with the numbers of winged aphids found on carrot plants (Tables 3.1 & 3.2). The suction trap captures, water trap captures and numbers of aphids found on the new carrots appear to be reflecting the same pattern. Winged aphids were present on the overwintered carrots well before they were captured in traps.

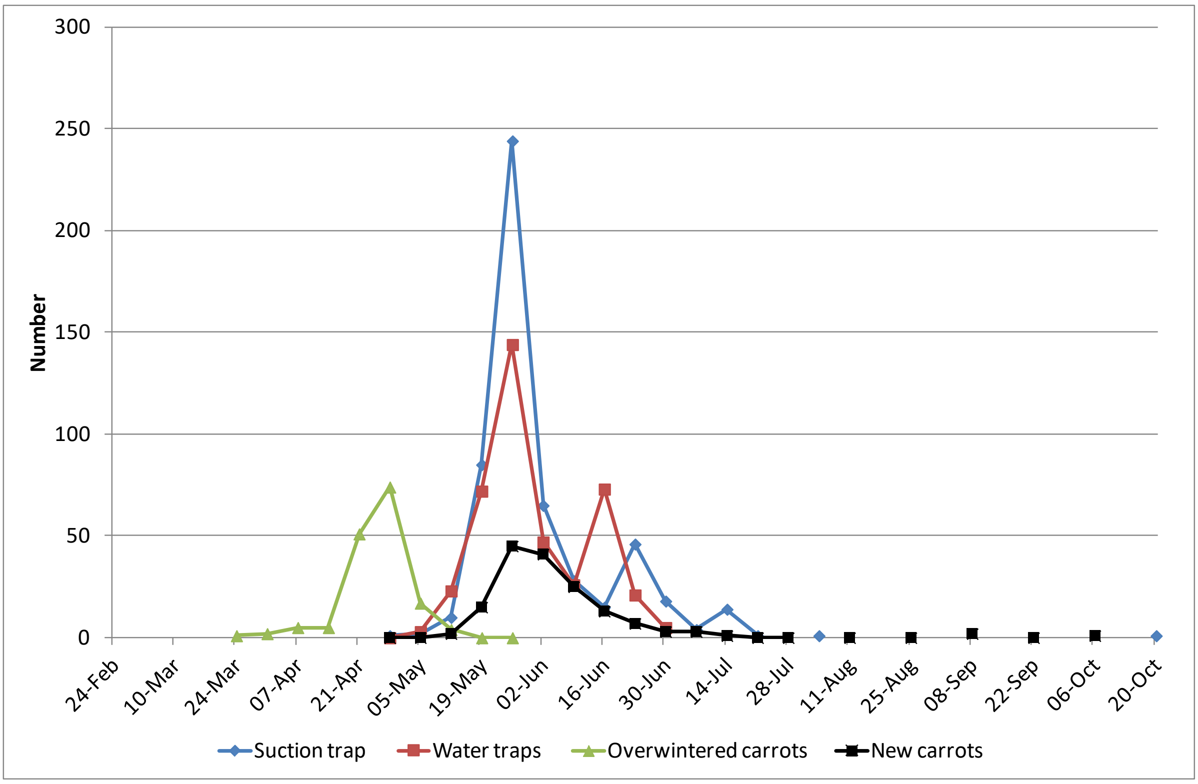

Figure 3.1 Numbers of winged willow-carrot aphid (C. *aegopodii)* captured in the suction trap, in the 4 yellow water traps in the field trial, on the plot of overwintered carrots and on plots of new carrots at Wellesbourne, Warwick in 2019.

Suction trap captures at Wellesbourne in 2016-2018 were also compared with water trap captures, with the water traps being placed close to plots of carrot. In these three years, willow-carrot aphids were always caught earlier in the yellow water traps than in the suction trap, by 1-2 weeks. Additionally peak numbers in the water traps were always one week after peak numbers were caught in the suction trap.

Field data 2021

Once again, at Wellesbourne, plots of carrots were maintained throughout the year to support the population of carrot fly. Numbers of aphids (primarily willow-carrot aphids) on these plots were monitored throughout the winter and spring 2020-21. Table 3.4 shows the numbers of aphids on the overwintered carrots from December 2020. Aphids were present from before the beginning of 2021 and numbers of winged and wingless aphids peaked in mid to late May, declining considerably by mid to late June. The numbers of parasitized aphids (aphid mummies) were also recorded.

Table 3.4 Numbers of aphids on 3 x 0.5 m lengths of row of overwintered carrots at Wellesbourne in 2020-21.

	Winged	**Wingless**	**Parasitised**
1-Dec-20	0.0	0.8	0.0
15-Dec-20	0.0	0.5	0.0
5-Jan-21	0.0	0.2	0.0
19-Jan-21	0.0	0.6	0.0
3-Feb-21	0.0	0.3	0.0
16-Feb-21	0.0	0.3	0.0
3-Mar-21	0.0	0.3	0.0
17-Mar-21	0.0	2.7	0.0
31-Mar-21	0.0	25.4	0.0
14-Apr-21	0.0	15.2	0.0
27-Apr-21	0.6	94.5	0.0
10-May-21	1.0	112.7	0.0
24-May-21	1.6	121.0	0.1
8-Jun-21	0.1	21.5	0.0
22-Jun-21	0.0	1.1	0.0
7-Jul-21	0.0	1.1	0.0

Aphids were also monitored on the new carrots sown in late March 2021 (Table 3.5). Winged aphids had arrived in the plots by 25th May and numbers of winged aphids peaked in early June. Numbers of wingless aphids peaked in mid-June and then declined over time, there being no aphids on the plants by late July. Numbers then started to increase again in late September. A small number of aphids were parasitised in June - July.

Table 3.5. Numbers of willow-carrot aphid on 3 x 0.5 m lengths of row of newly-sown carrots (March) at Wellesbourne in 2021.

	Winged	Wingless	Parasitised
25-May-21	0.2	0.3	0.0
1-Jun-21	0.3	3.4	0.0
8-Jun-21	0.8	14.3	0.0
15-Jun-21	0.2	48.9	0.1
22-Jun-21	0.0	6.1	0.0
29-Jun-21	0.0	2.2	0.1
7-Jul-21	0.0	1.2	0.1
14-Jul-21	0.0	0.2	0.0
20-Jul-21	0.0	0.1	0.1
27-Jul-21	0.0	0.0	0.0
16-Aug-21	0.0	0.0	0.0
1-Sep-21	0.0	0.0	0.0
28-Sep-21	0.0	0.1	0.0
12-Oct-21	0.0	0.2	0.0

10-Nov-21	0.0	1.2	0.0
23-Nov-21	0.0	0.7	0.0
8-Dec-21	0.0	0.3	0.0
26-Oct-21	0.0	0.2	0.0

Summary data for willow-carrot aphid from the network of Rothamsted Suction traps are shown in Table 3.6.

Table 3.6. Summary of captures of willow-carrot aphid in 2021 by the network of suction traps run by Rothamsted Research and SASA (from the weekly bulletins).

Cavariella aegopodi	Inverness	Dundee	Edinburgh	Ayr	Newcastle	FERA, York	Preston	Kirton	Broom's Barn	Wellesbourne	Hereford	Rothamsted	Writtle	Ascot	East Malling	Starcross	Total
Week ending																	
11-Apr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
18-Apr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
25-Apr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
02-May	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	**1**
09-May	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	**2**
16-May	0	0	0	0	0	1	9	2	1	1	6	2	5	8	4	4	**43**
23-May	0	1	36	0	0	9	2	0	15	82	5	2	3	12	12	5	**184**
30-May	0	5	87	0	1	62	108	15	86	235	84	141	68	106	30	31	**1059**
06-Jun	0	44	245	0	11	98	210	359	347	998	375	272	182	229	125	97	**3592**
13-Jun	1	86	103	0	4	89	58	328	253	519	152	294	100	244	80	167	**2478**
20-Jun	0	6	17	0	3	6	41	85	60	188	64	78	34	69	21	37	**709**
27-Jun	0	1	2	4	1	22	13	8	12	57	0	14	18	35	9	21	**217**
04-Jul	40	0	3	0	0	9	28	3	6	30	18	6	7	0	1	14	**165**
11-Jul	17	3	7	0	3	3	16	3	7	6	8	0	1	1	0	2	**77**
18-Jul	2	4	1	0	1	10	14	2	7	3	2	4	0	0	0	1	**51**
25-Jul	3	2	1	0	0	4	4	1	0	3	2	1	0	0	0	1	**22**
01-Aug	0	0	5	0	0	4	0	0	0	0	0	0	1	0	0	2	**12**
08-Aug	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	**2**
15-Aug	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	**2**
22-Aug	3	0	3	0	0	0	0	1	0	0	0	0	0	0	0	0	**7**
29-Aug	0	5	0	0	0	4	1	0	0	0	1	0	0	0	0	1	**12**
05-Sep	0	8	0	0	0	9	0	0	1	1	0	0	0	0	0	0	**19**
12-Sep	0	4	1	0	0	28	1	3	2	0	1	0	0	0	0	0	**40**
19-Sep	0	0	1	0	3	134	0	4	24	8	0	1	1	0	0	0	**176**
26-Sep	0	7	0	0	0	1403	0	51	82	6	0	0	0	1	1	0	**1551**
03-Oct	0	0	0	0	0	126	9	2	3	0	0	0	0	0	0	0	**140**
10-Oct	0	0	0	0	0	9797	151	386	388	17	1	1	15	1	2	1	**10760**
17-Oct	0	0	0	0	0	1544	12	19	107	15	0	1	6	1	2	1	**1708**

Figure 3.2 compares the pattern of captures of willow-carrot aphid in the suction trap and the water traps at Wellesbourne in 2021 with the numbers of winged aphids found on carrot plants (Tables 3.4 & 3.5). The suction trap captures, water trap captures and numbers of aphids found on the new carrots appear to be reflecting the same pattern. As in 2019, winged aphids were present on the overwintered carrots well before they were captured in traps

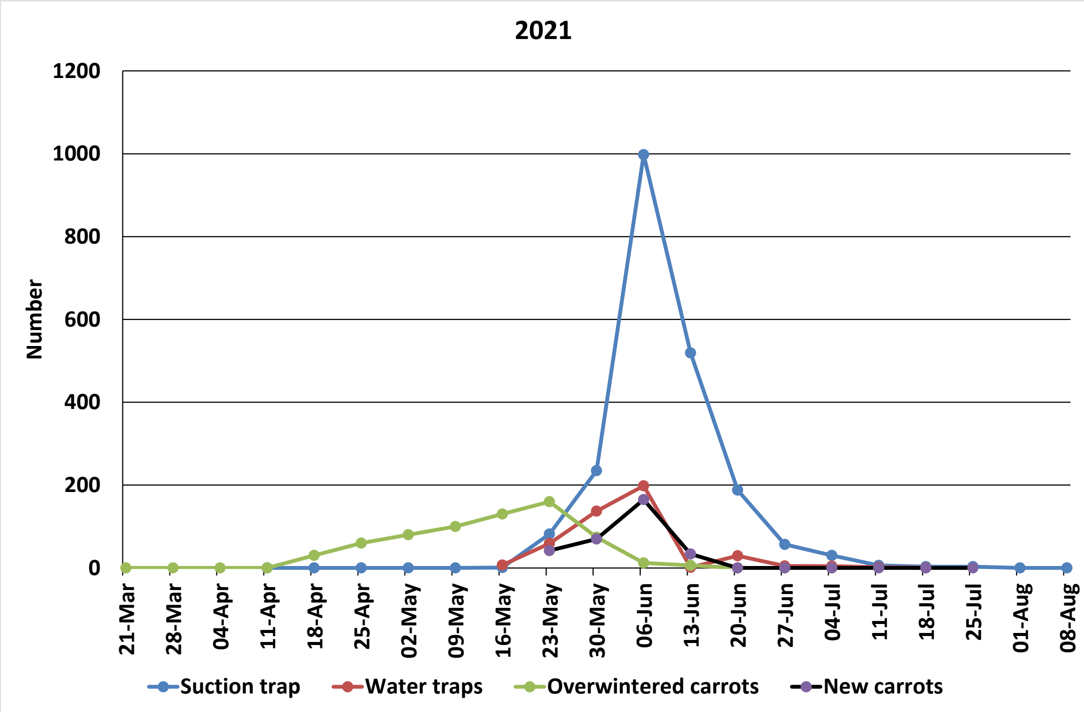

Figure 3.2 Numbers of winged willow-carrot aphid (C. *aegopodii)* captured in the suction trap, in the 4 yellow water traps in the field trial, on the plot of overwintered carrots and on plots of new carrots at Wellesbourne, Warwick in 2021.

3.2 Relationships between suction trap data and weather data

A larger set of suction trap data than available originally has been used to refine the day-degree model for willow-carrot aphid. To predict the dates of first and 10% capture, the day-degree sums are 325 and 451 day-degrees respectively from 1 February above a base temperature of 4.4°C. It seems to make little difference to 'accuracy' whether the start date is 1st January or 1st February (although the day-degree sums differ) or whether the base temperature is 4.4 or 4°C (the day-degree sums again differ).

Suction trap data for the parsnip aphids is more limited, partly because they are often less abundant than willow-carrot aphid. Despite the fact that the parsnip aphids are thought to have similar life-cycles to willow-carrot aphid there does not seem to be a 'constant' relationship between the dates of first or 10% capture in suction traps and accumulated day-degrees. The same is true for *M. persicae* (which is not unexpected since it has a different method of overwintering – as mobile aphids rather than cold-resistant eggs on a woody host). For *M. persicae,* the established way to forecast the spring migration is the relationship between the date of first capture etc. with the mean air temperature in January - February, used by the Rothamsted Insect Survey to produce forecasts in early March each year. Using a similar approach for the parsnip aphids produced some statistically-significant relationships but these were not as robust as the day-degree forecast for willow-carrot aphid (this may be partly because there is less data).

3.3 Day-degree forecasts

The revised day-degree model using accumulated day-degrees from 1 February predicted the start of willow-carrot aphid flight activity at Wellesbourne in 2019 to be on 23 April (Figure 3.3) (when 325 day-degrees above a base of 4.4°C had been accumulated). The first aphid was captured in the Wellesbourne suction trap by 28 April and in water traps by 7 May (samples taken weekly). The forecast date for 10% of the migration was 16h May.

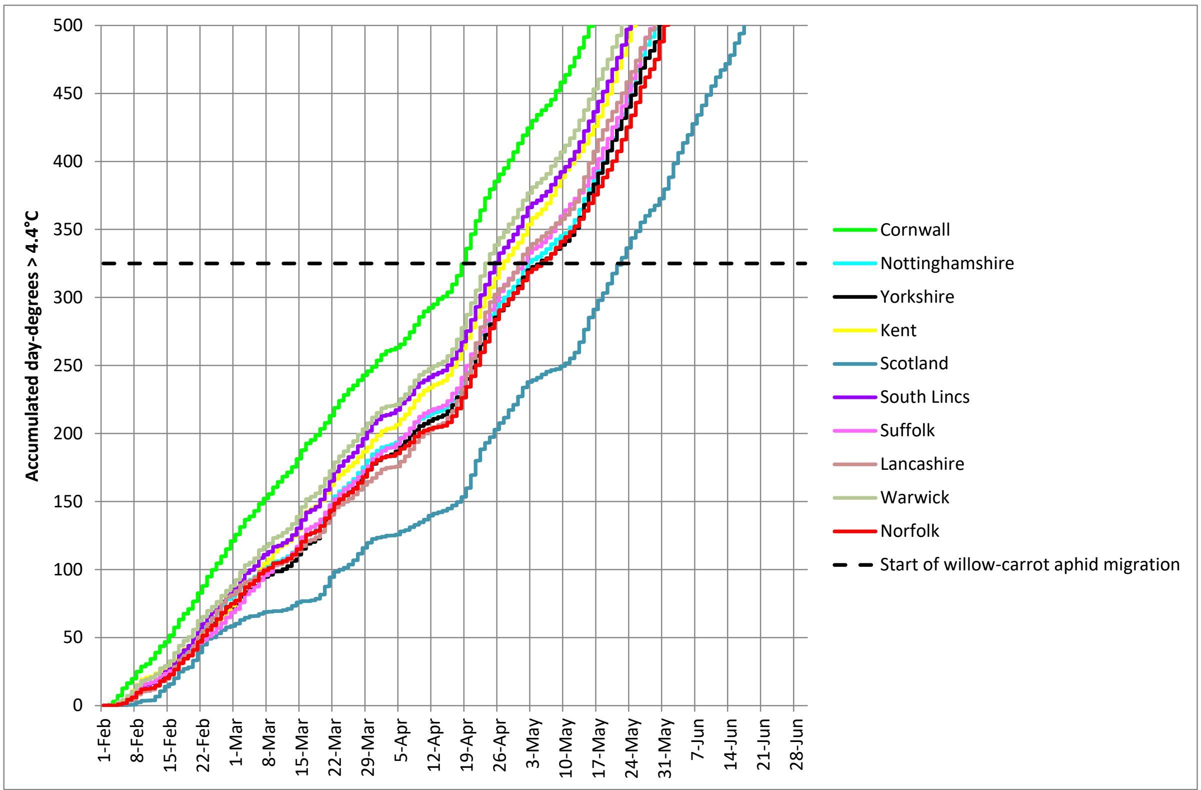

Figure 3.3. Day-degree forecasts for 2019 for the start of the willow-carrot aphid 'migration' to susceptible crops. The migration is forecasted to begin when 325 day-degrees above 4.4°C have been accumulated from 1 February.

The same day-degree model predicted the start of willow-carrot aphid flight activity at Wellesbourne in 2021 to be on 9 May (Figure 3.4) (when 325 day-degrees above a base of 4.4°C had been accumulated). The first aphid was captured in the Wellesbourne suction trap by 16 May and in water traps by 18 May (samples taken weekly). The forecast date for 10% of the migration was 29 May.

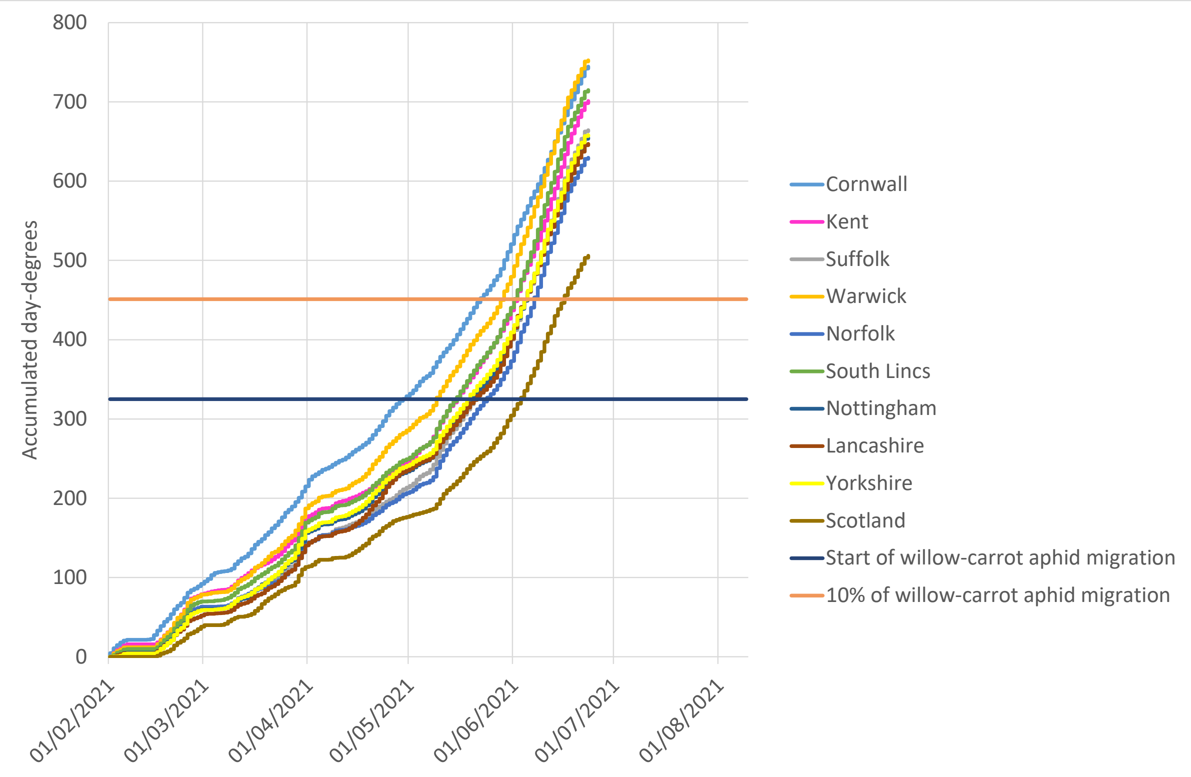

Figure 3.4. Day-degree forecasts for willow-carrot aphid in 2021. Information from the Rothamsted Suction trap captures have been used to estimate the mean number of D° from 1 February until the first aphid of the year is caught in a suction trap (the start of the migration to carrot) and when 10% of aphids are caught. This is after approximately 325 and 451D° respectively.

3.4 Comparisons between years

Figure 3.5 compares suction tap captures at Wellesbourne in 2019 and 2021, confirming that willow-carrot aphids were more abundant in 2021 than 2019 but that the migration was earlier in 2019.

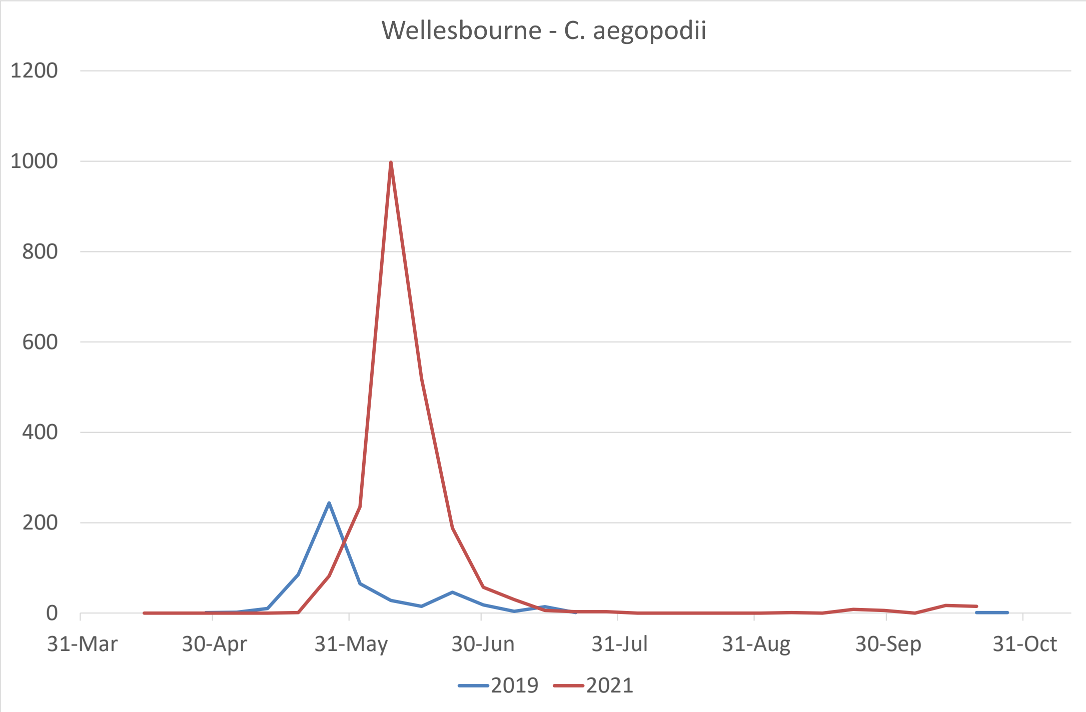

Figure 3.5 Suction tap captures at Wellesbourne in 2019 and 2021, confirming that willow-carrot aphids were more abundant in 2021 than 2019 but that the migration was earlier in 2019.

Table 3.7 compares 2019 and 2021 with regard to the timing of the migration of willow-carrot aphids at Wellesbourne. The migration was approximately 2 weeks later in 2021.

Table 3.7 Comparisons between 2019 and 2021 regarding the timing of the migration of willow-carrot aphids at Wellesbourne.

	2019	**2021**
Forecast start of migration	23 April	9 May
Date by which first aphid captured in suction trap (weekly samples)	28 April	16 May
Date by which first aphid captured in water traps (weekly samples)	7 May	18 May
Forecast 10% migration	16 May	29 May

3.5 Forecast refinement/validation

Fera Science Ltd have a very large historical data set on aphid captures in yellow water traps in commercial crops (2004-2018) and this was sent to Warwick to see if the data could be used for forecast validation. The data set is quite 'fragmented' and there appears to be no information about when trapping started and finished and so it is possibly of limited use for forecast validation (there are no dates with zero captures). A small sample of the data set is presented in Figures 3.6-3.8 and in this case the data for a region have been plotted on the same graph as the data from the nearest suction trap - as a scatter plot. Data for the Midlands and East Anglia appear to 'fit' with suction trap catches but for Grampian the water trap catches seem later than the captures in the suction trap at Dundee (the nearest functioning suction trap).

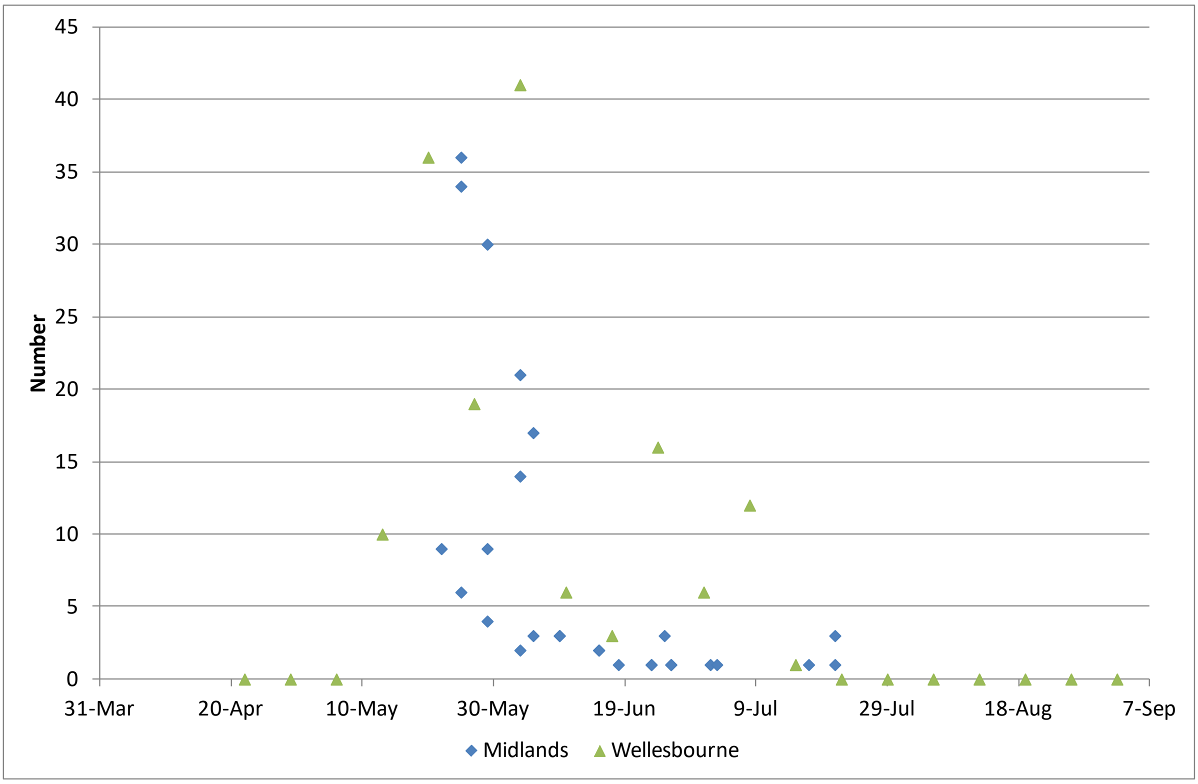

Figure 3.6 Scatter plot comparing data from Fera Science Ltd water trap samples in the Midlands region in 2018 with suction trap data from Wellesbourne.

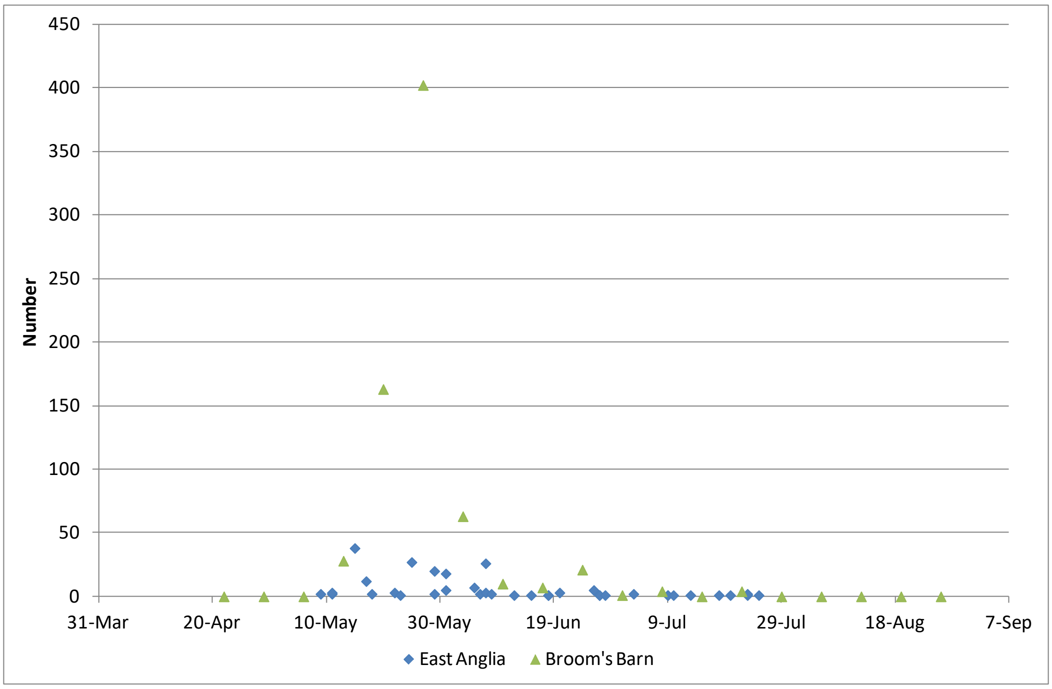

Figure 3.7. Scatter plot comparing data from Fera Science Ltd water trap samples in the East Anglia region in 2018 with suction trap data from Broom's Barn.

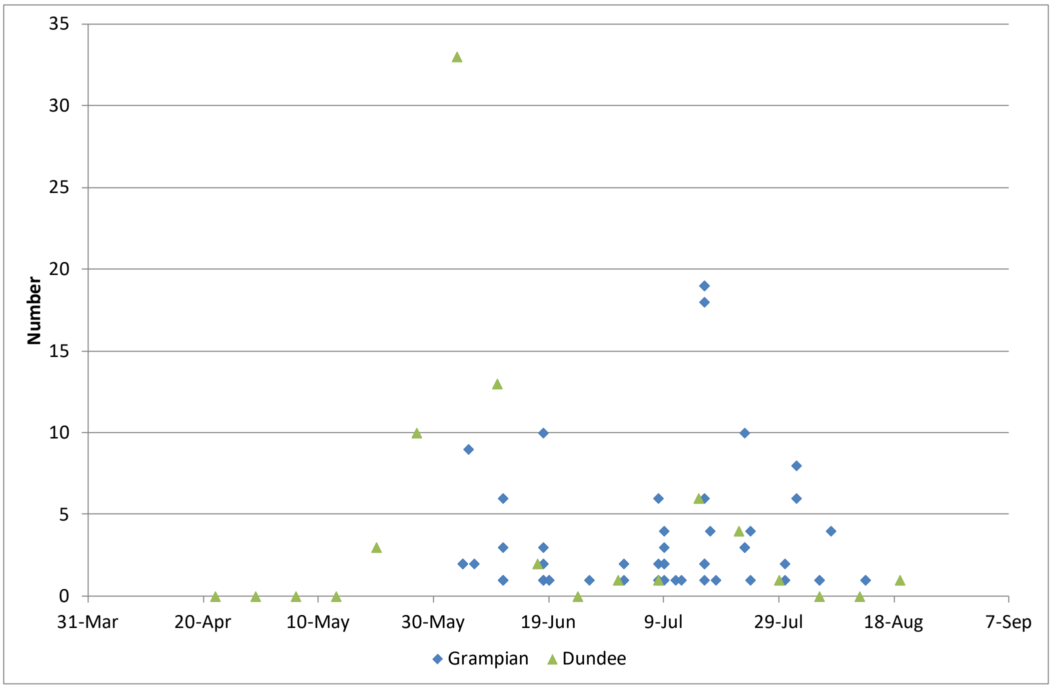

Figure 3.8. Scatter plot comparing data from Fera Science Ltd water trap samples in the Grampian region in 2018 with suction trap data from Dundee.

3.6 Information available to growers

Throughout the project, including as far as feasible in 2020 (Covid pandemic), information on aphid activity relevant to carrot crops has been available as part of the AHDB Pest Bulletin, hosted in 2019-2021 on the Syngenta UK web site. This has included outputs from the day-degree forecasts, suction trap counts and plant monitoring data at Wellesbourne.

In 2020 and 2021, the Fera/AHDB potato water trap data sets were made available to the AHDB Pest Bulletin on a weekly basis, providing additional information on aphid activity. Figure 3.9 shows the summarised data for 2021.

In 2021, the aphid forecast was developed by AHDB into a forecasting tool that is hosted on the AHDB Horticulture web site Willow-carrot aphid migration forecast tool | AHDB.

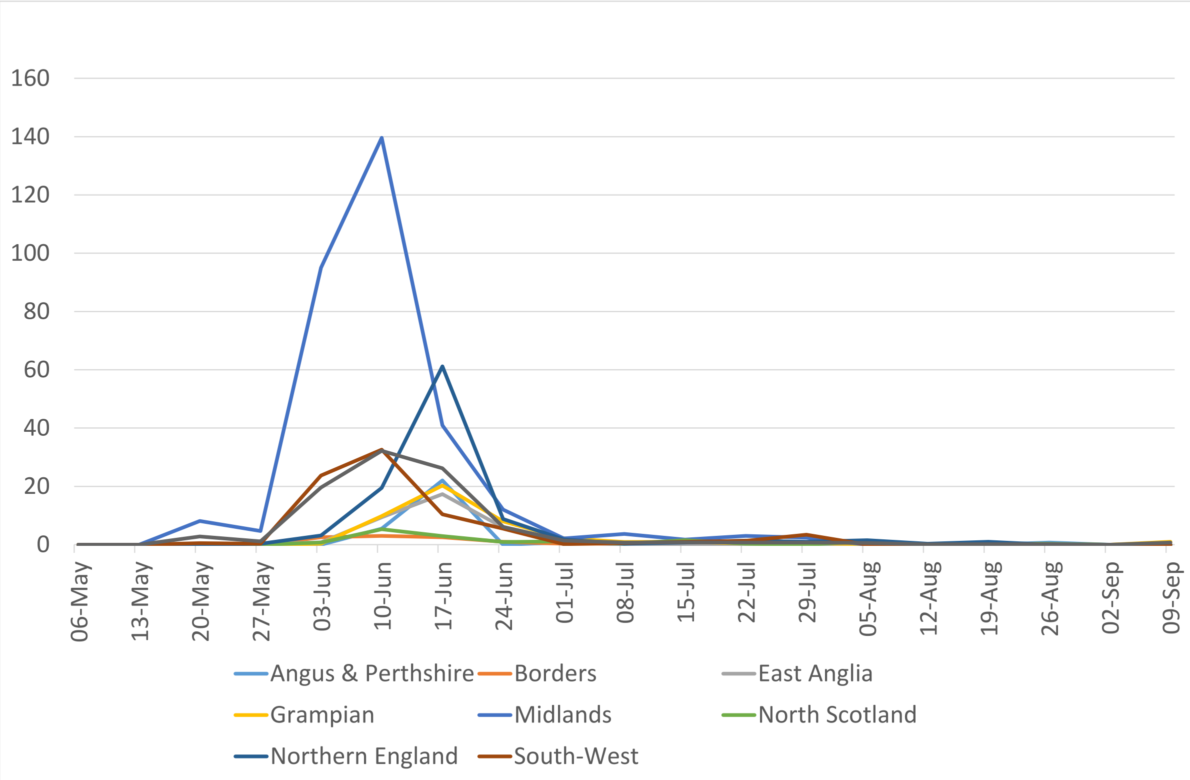

Figure 3.9. Data (numbers of aphids) summarised by region from the Fera/AHDB potato water trap data sets for 2021 that were made available to the AHDB Pest Bulletin on a weekly basis.

Discussion

These field transmission data presented in this report represent the first two years of a three-year project. Trials were conducted in 2019, and year two was delayed to 2021 due to the impact of COVID on the abilities of project partners to conduct field work. In both years there has been a marked difference in the incidence of virus recorded between the two field trial sites. Whilst the reasons for this are unclear, regional differences in virus incidence have been recorded previously in carrot crops (AHDB FV382b), with notable differences in field incidence of a range of carrot viruses recorded between North Yorkshire and Norfolk in that project. This is also a phenomenon noted in numerous virus surveys. Within the previous carrot virus work local differences in virus incidence were also recorded, suggesting that within a region virus incidence will also be influenced by local context. However, although localised influences on virus incidence also been reported in other crop pathosystems such as grasses/cereal yellow dwarf virus (Borer et al. 2010) there is little understanding of the factors driving these local influences. The Stamford Bridge site from 2019, and the Buttercrambe site from 2021 were situated in an area where both previous carrot virus research (FV382b) and local knowledge from the grower suggested a risk of virus. The Warwick site, by comparison is a long-term field trial site and is known to have had high levels of virus transmission from previous trials. The aphid flights at both sites had a peak in the middle of the trial period (28th May) during 2019, with the main aphid species present in traps through that period being *C. aegopodii*. Whilst virus transmission didn't track flights of this particular species throughout the season, it appears from these raw data that *C. aegopodii* may be the key aphid species driving transmission of CtRLV in the early part of the growing season. In 2021 there was a late peak of aphids in the Buttercrambe trial (13 July), which did not directly correspond with peaks recorded at Warwick (8 June, 22 June and 6 July). However the numbers of aphids caught throughout the trial at York were exceptionally low throughout the season which explains the virus transmission which at best/worst was 1% virus in a week.

At both sites CtRLV was the detected most commonly in 2019, with CYLV detected in only a single finding in a single week at Stamford Bridge, and sporadic detections at Warwick, found in six of the 10 weeks of the trial, and in all but one week these were individual findings, except for the week of 25 June where 2 bulked samples tested positive for the virus. This is a little unexpected as the results of AHDB FV382b suggested that CYLV may be present at as high an incidence as CtRLV. However, this may again be the result of local differences, as in that project a greater relative incidence of CYLV was recorded in Yorkshire than in Norfolk. However, this may also be a result of the experimental set-up. The previous field work was based upon a single sample, taken mid- summer (Late June), and as such was a 'snapshot' of virus health in the crop. Samples were also taken more broadly from across the fields, and not limited to small pre-selected plots. However, based upon prior knowledge placing sites toward the field margin should maximise the chance of detecting CYLV transmission should it occur (Fox et al. 2017b). If CYLV has a different virus-vector-host relationship to CtRLV, which is likely, there are multiple factors which could influence the timing of transmission not least source plant species and potential range of vector species. At the Warwick site in 2021 CtRLV was again most commonly detected, with low incidence (<5%) transmission of CYLV early in the season and only during peak transmission periods of CtRLV (01 June -08 June). Given these CYLV transmission periods coincide with a peak of willow carrot aphid it is likely that, for this site at least, CYLV is being transmitted by this aphid. The late season transmission peak (06 July) of CtRLV did not correspond to catches of willow-carrot, however, there was a small underlying peak of parsnip aphid which, combined with a build up of inoculum in the surrounding carrots, may account for this late transmission. This will be further investigated during the 2022 season control trials through ongoing testing to monitor timing of transmission and aphid movements.

Comparisons of monitoring data collected in different ways (plant sampling, suction traps, water traps) suggest that all approaches are broadly measuring the 'same thing'. There are some details of the biology of all species of *Cavariella* that it would be helpful to explain but this is probably outside the scope of this project. Several species of aphid undoubtedly can overwinter on suitable host crops provided conditions are appropriate and this has been known for some time. However, what is not known is what contribution these aphids make in terms of virus transmission to new crops. At Wellesbourne, aphids are found regularly on overwintered carrots and in 2019 they were present when sampling started on 21st March. These aphids had all but disappeared before willow-carrot aphids were captured in the suction trap at Wellesbourne, possibly due to predators (ladybirds), parasitiods and the increasingly poor condition of the plants. That the 'early' winged aphids were not detected by the suction trap is not surprising since overall they were probably a very small and localised population. Large areas of carrot might provide a different story.

Of interest also is what happens to aphid infestations in carrot crops. New carrots at Wellesbourne are invariably colonised by winged aphids who produce wingless young but in most years the infestation declines after a few weeks, again possibly due to natural enemies. It is not clear what happens in commercial crops where insecticide pressure is likely to be greater, which may impact negatively on natural enemies.

The aim of the work on monitoring and forecasting is to improve decision support for growers. The day-degree forecast for willow-carrot aphid appears to be relatively robust, whereas it may be more difficult to forecast the activity of *M. persicae* and the parsnip aphids. If this project can 'confirm' that willow-carrot aphid is the key species transmitting virus then this will make the provision of warnings simpler. The results from Warwick in 2021 indicate that parsnip aphid may play a role in late season transmission, however the importance of this effect over a season will be further considered during the final year control trials at Warwick.

Conclusions

- Greater virus transmission was recorded in the trials at Warwick than at Stamford Bridge (2019) or Buttercrambe (2021)
- The trials at Stamford Bridge at Buttercrambe did not show a good relationship between aphid flights and virus in either 2019 or 2021
- The trials at Warwick had greater incidence of virus transmission throughout both seasons.
- Most virus detected was carrot red leaf virus at both sites, with CYLV being occasionally detected throughout the season.
- Transmission appears to track movements of *Cavariella aegopodii,* but this will be further refined in the coming season.
- Comparisons of monitoring data collected in different ways (plant sampling, suction traps, water traps) suggest that all approaches are broadly measuring the 'same thing'.
- The day-degree forecast for willow-carrot aphid appears to be relatively robust, whereas it may be more difficult to forecast the activity of *M. persicae* and the parsnip aphids.
- For 2022 the focus of the trial will now change to look at control strategies through a spray trial conducted at Warwick with Fera providing diagnostic support.

Knowledge and Technology Transfer

The following activities have been used to promote the project. Further opportunities will be pursued in 2020-2021 as the project gathers further data.

- Article for Grower Magazine
- Poster at Onion and Carrot growers' conference (November 2019)
- Meeting of IOBC Working Crop on 'Integrated Protection of Field Vegetables' (October 2019) - mentioned the project in the context of decision support
- AAB meeting Advances in Biological Control and IPM 2019: Addressing the innovation crisis (November 2019) – mentioned the project in the context of decision support.

- EUVRIN IPM Working Group meeting (November 2019) – mentioned the project in the context of decision support.
- Keeping track of pests. AAB Meeting, November 2021
- Pests of carrot with a focus on aphids and virus. Warwick Crop Centre Webinar: Carrots - diseases, pests and genetic resources 14th March 2022

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