Project title:	**Investigating the timing of transmission of carrot viruses to improve management strategies**
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Project leader:	Dr Adrian Fox, Fera Science Ltd
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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.

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GROWER SUMMARY

Headline

Research has identified the main vectors and timing of transmission of carrot red leaf virus. Transmission of carrot red leaf virus appears to track well with flights of willow-carrot aphid. A vector control trial suggests early season control is key to mitigating against yield loss from aphid transmitted virus in carrots. A day-degree forecast for willow-carrot aphid appears to produce useful information on timing for growers.

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 focused 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 was 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 was 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 Field trial (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.

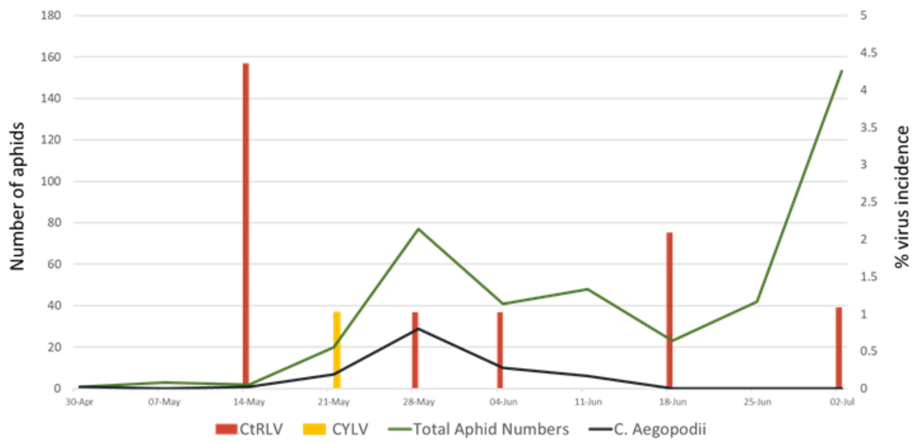

Figure1. Showing the limited virus transmission recorded at Stamford Bridge, Yorkshire. Virus content in plots is shown in the bars (Red for CtRLV, yellow for CYLV), and aphid flight data in the lines on the graph (Green for total aphid flights, Black for willow-carrot aphid).

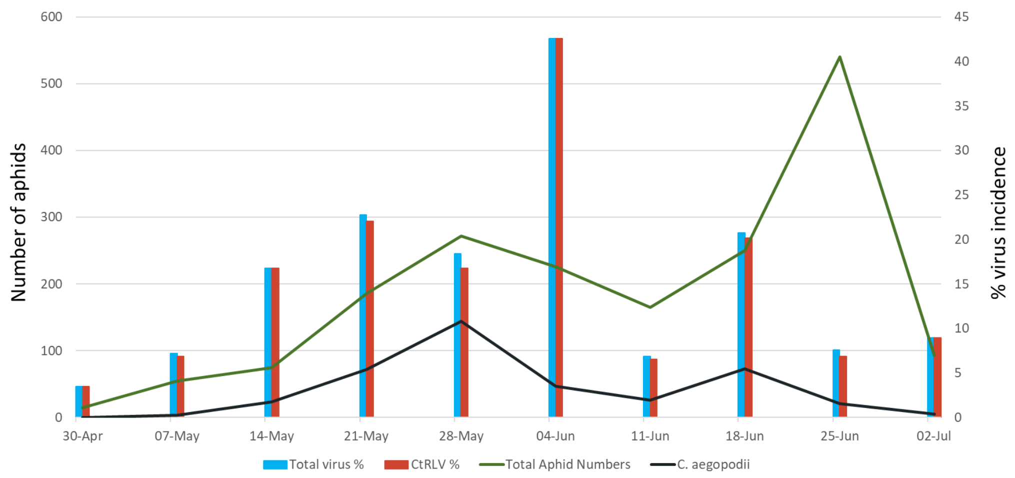

Figure 2. Virus transmission recorded in trial plots at Warwick University. Virus content is shown in the bars (Blue for total virus content, Red for CtRLV), and aphid flights in the lines on the chart (Green for total aphids, Black for willow-carrot aphid)

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 Field trial (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 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 captures 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 does not correspond with a rise in numbers of willow-carrot aphid. However, during this period there was a rise in the captures of *C. pastinaceae* (parsnip aphid) representing a large proportion of the small peak in aphid captures 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.

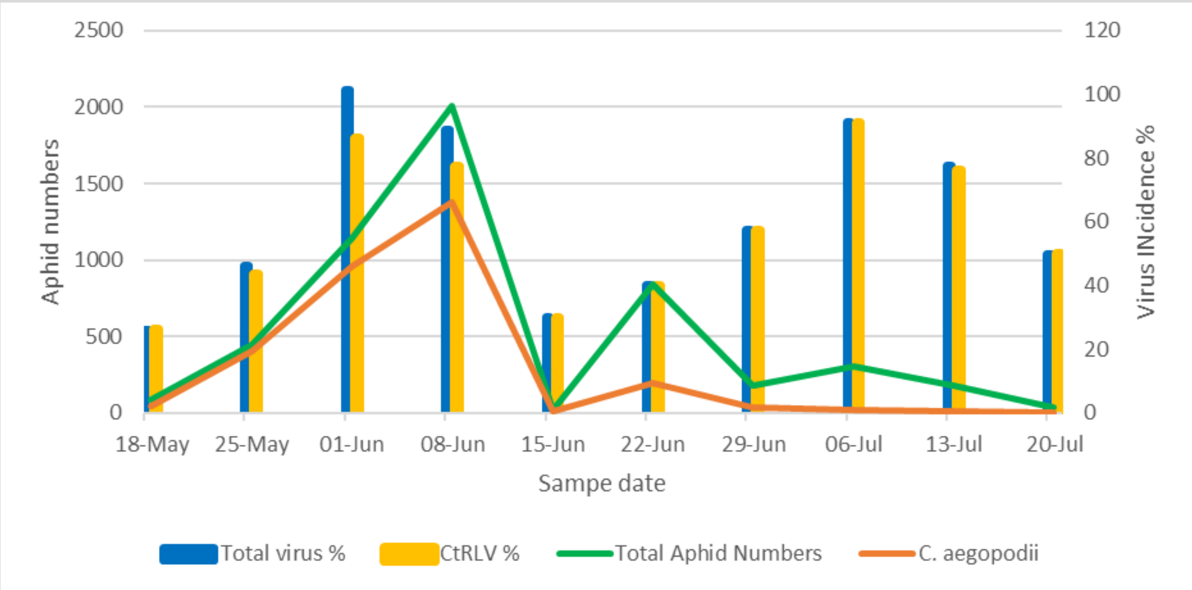

Figure 3. Virus transmission recorded in trial plots at Warwick University. Virus content is shown in the bars (Blue for total virus content, Yellow for CtRLV), and aphid flights in the lines on the chart (Green for total aphids, Orange for willow-carrot aphid)

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*).

Year 3 (2022) Vector management trial

The final year of the programme of research switched from investigating the timing of transmission to focus on the control of the vector *C. aegopodii*, the willow carrot aphid. The trial combined currently available and near-market products to investigate their efficacy at controlling both virus infection and disease impact from the virus, including foliar and root symptoms and yield reduction. Throughout the trial transmission of CYLV was below levels needed for reliable detection at the sampled rate, and consequently the focus of the results reported here are on CtRLV.

The treatments were conducted over a 10 week period (9 treatment dates). The treatments and the dates of specific applications are presented in Table 1. First treatment date was on 9 May (T1) and final treatment on 6 July 9 (T9). Peak aphid populations in the trial were recorded the following week, with both willow-carrot aphid and peach-potato aphid numbers peaking in the week of 19 May (week 2 of the trial) and reducing through the period to 16 June (week 6 of the trial) (See section 4.3). Virus content in the untreated plots was monitored through weekly sampling, starting 3 weeks after T1. From the first sampled week 18 of the 20 bulked samples tested were positive for CtRLV (calculated virus content 36%, CI: 20.53-58.47). From the third sampling week all bulked subsamples were positive for CtRLV (calculated virus content 100% CI: 29.97-N/A), indicating the high virus pressure in the initial weeks of the trial.

Table 1. Treatment programmes trialled in spray control trial

Treatment	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
Timing [1]		**7DAT1**	**7DAT2**	**7DAT3**	**7DAT4**	**7DAT5**	**7DAT6**	**7DAT7**	**7DAT8**
Date	09-May	17-May	25-May	31-May	07-Jun	14-Jun	20-Jun	28-Jun	06-Jul
1	Untreated	Untreated	Untreated	Untreated	Untreated	Untreated	Untreated	Untreated	Untreated
2	Movento 0.3	Movento 0.3	Movento 0.3	Movento 0.3	Movento 0.3	Movento 0.3	Movento 0.3	Movento 0.3	Movento 0.3
3	Movento 0.3		Teppeki 0.14		Movento 0.3		Teppeki 0.14		
4	Teppeki 0.14		Movento 0.3		Teppeki 0.14		Movento 0.3		
5	Gazelle 0.2		Movento 0.3		Teppeki 0.14		Movento 0.3		Teppeki 0.14
6	Movento 0.3		Gazelle 0.2		Teppeki 0.14		Movento 0.3		Teppeki 0.14
7	Movento 0.3	Teppeki 0.14	Movento 0.3	Teppeki 0.14					
8	Movento 0.3		Movento 0.3						
9	Coded 0.25		Coded 0.25						
10	Teppeki 0.14		Teppeki 0.14						
11	Gazelle 0.2		Gazelle 0.2						
12 [2]	Minecto One		Minecto One						

1- 7DATX represents “days after treatment”
2- Minecto One – only carrot fly control is specified on the label.

The prevalence of virus in plots was measured at the mid- and end- points of the trial (Figure 4). Given the lag-time allowed for the bio-amplification of virus within plants to reach detectable levels the “week 5” and “week 10” sample points were three weeks in arrears of the actual treatment weeks. At week 5, all treatment programmes showed a reduction in virus content by comparison to the untreated control to approximately half of the virus content of untreated plots. Some treatments, showed little increase in virus content over the later half of the trial, including the regimes with Movento and Teppeki in the earliest treatment. However, the two programmes with early Gazelle treatments had a marked increase between the mid-point virus content and the virus prevalence recorded at the end of the trial.

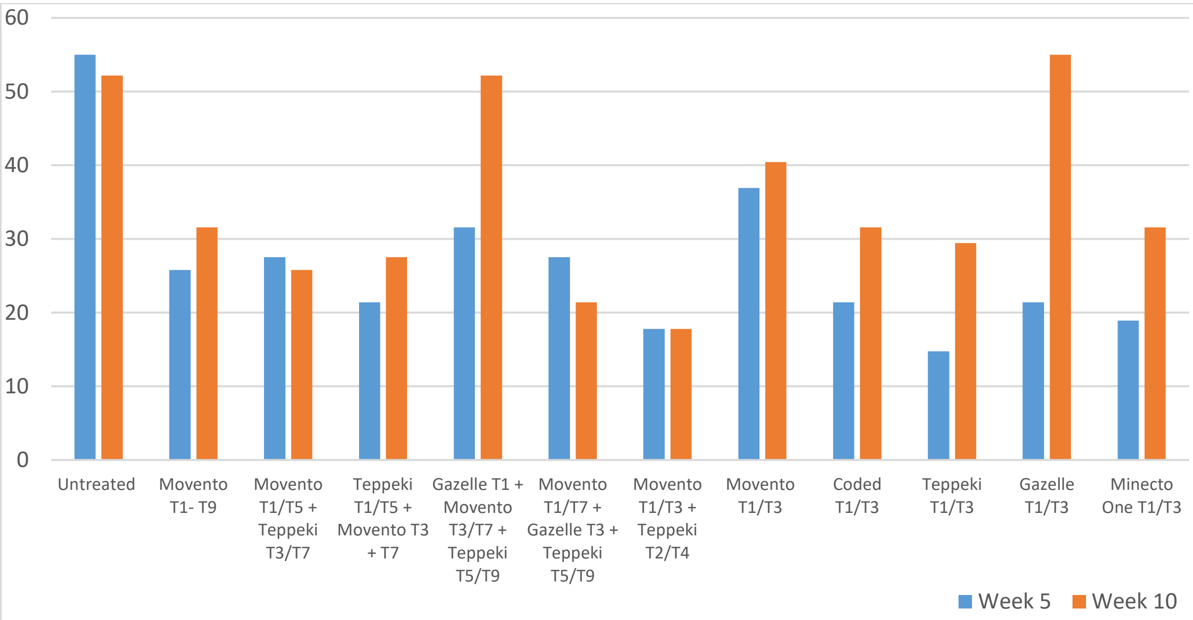

Figure 4. % virus content recorded in plots of virus treatment

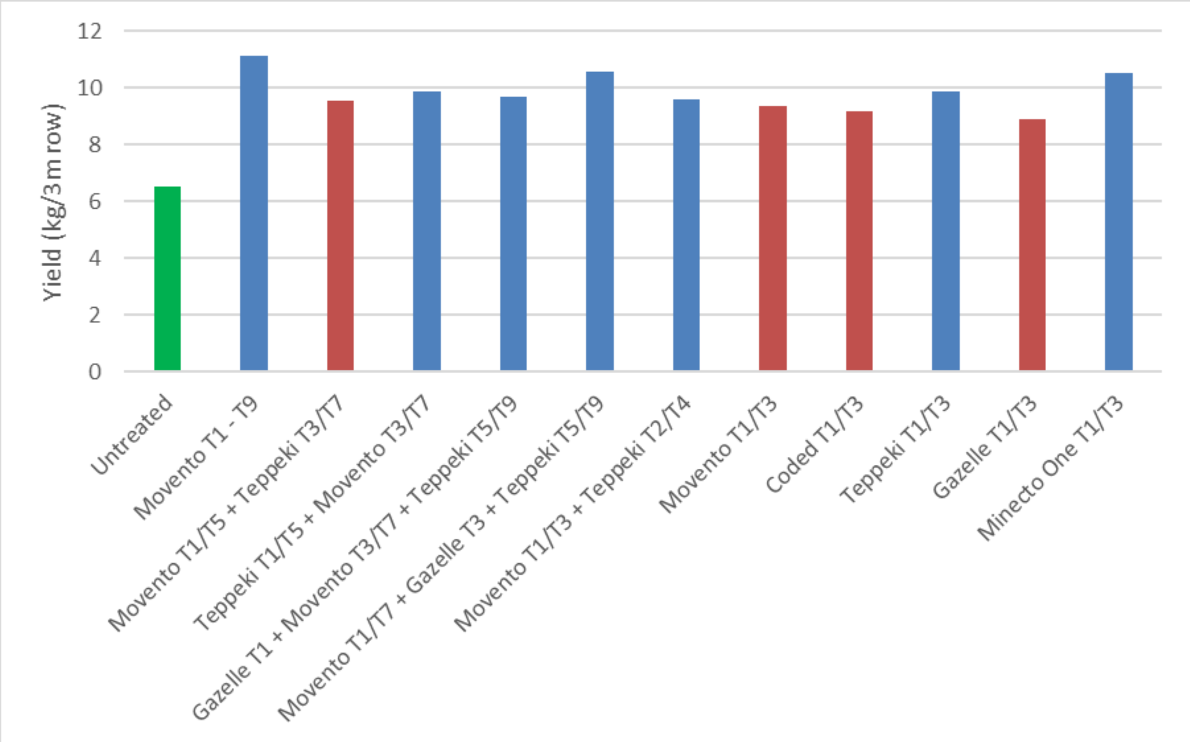

Figure 5. Yield of carrot roots, presented as Kg per 3 m row. Green denotes "untreated control", red where the reduction in yield was significantly reduced from treated control (Movento T1-T9).

The application of chemical controls had a positive impact on foliar symptom development with all treatments (See section 4.3). Similarly, all treatments had the effect of mitigating against yield loss (Figure 5). Although there were little differences between treatments, the yield from some programmes were significantly lower than the treated control (intensive Movento treatment). However there does not appear to be a correlation between the virus content at mid- and end- point and the impact on yield within the trial.

Comparison of methods of monitoring aphid infestations (on plants, suction trap, water traps)

At Wellesbourne, plots of carrots are maintained throughout the year to support the population of carrot fly. The carrots are overwintered, sometimes under covers, and then uncovered. New plots of carrots are sown in late March and then in May each year. Numbers of aphids (primarily willow-carrot aphids) were monitored on these plots throughout each year by counting the number of aphids on a fixed length of row or a fixed number of plants. Records were taken of the numbers of winged, wingless and parasitised aphids. Winged aphids were also monitored in the Rothamsted Insect Survey suction trap located at Wellesbourne and in water traps in the field trials in 2019, 2021 and 2022 – as above. All these data sets were compared.

Figure 6 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. The suction trap captures, water trap captures and numbers of aphids found on the new carrots appear to be reflecting the same pattern. However, winged aphids were present on the overwintered carrots well before they were captured in traps. The same pattern was shown in all three years (2019, 2021, 2022).

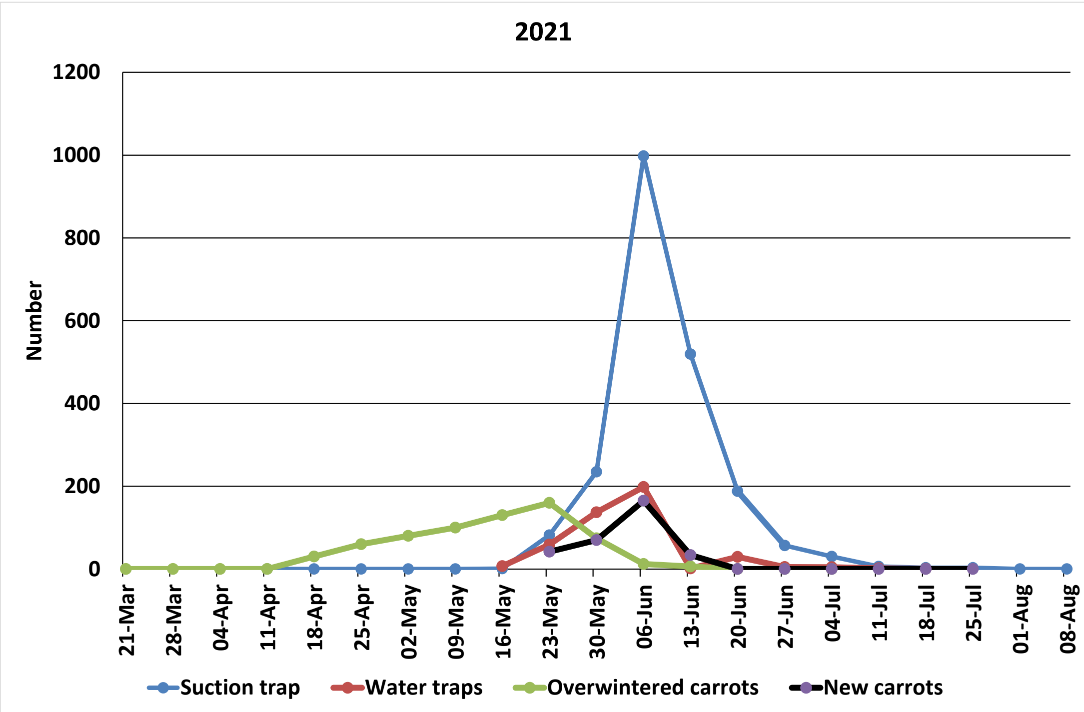

Figure 6. Numbers of winged willow-carrot aphid (C. *aegopodii)* captured in the suction trap, in the 4 yellow water traps in the field trial, on the plot of overwintered carrots and on plots of new carrots at Wellesbourne, Warwick in 2021.

Relationships between suction trap data and weather data

A larger set of suction trap data than available originally was 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 1 January or 1 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).

Day-degree forecasts

The revised day-degree model using accumulated day-degrees from 1 February was used to predict the start of willow-carrot aphid flight activity at Wellesbourne in each year. This information was also provided to growers in real time through the Pest Bulletin. An example of the Pest Bulletin information (for 2022) is shown in Figure 7.

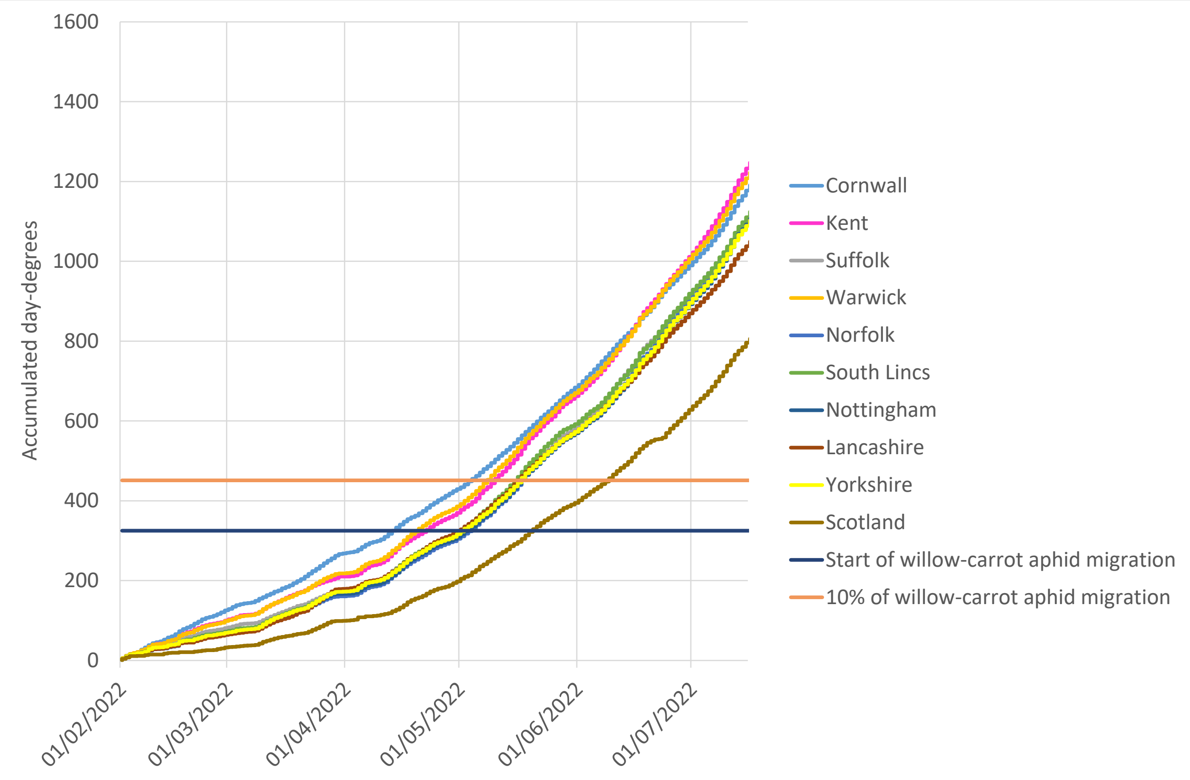

Figure 7. Day-degree forecasts for willow-carrot aphid in 2022. Information from the Rothamsted Suction trap captures have been used to estimate the mean number of D° from 1 February until the first aphid of the year is caught in a suction trap (the start of the migration to carrot) and when 10% of aphids are caught. This is after approximately 325 and 451D° respectively.

Comparisons between years

Figure 8 compares suction trap captures at Wellesbourne in 2019, 2021 and 2022, confirming that willow-carrot aphids were most abundant in 2021 but that the migration was earliest in 2022.

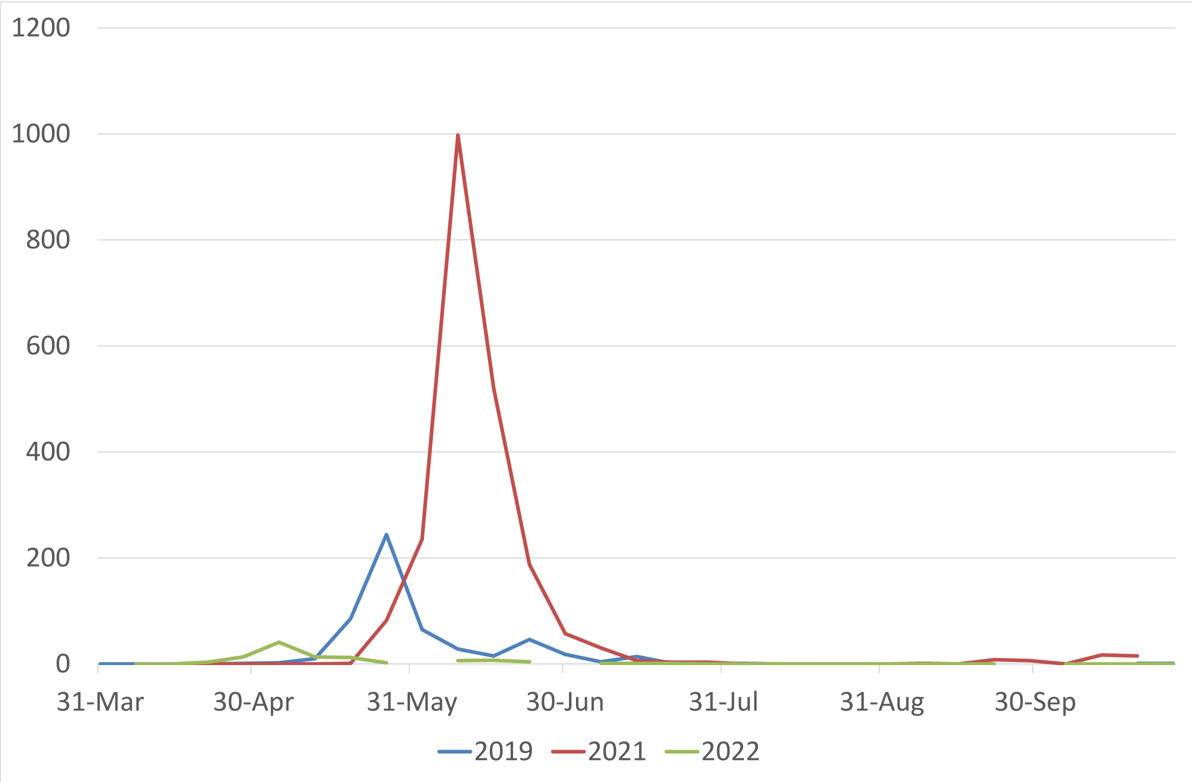

Figure 8. Suction tap captures (number of aphids) at Wellesbourne in 2019, 2021 and 2022 confirming that willow-carrot aphids were more abundant in 2021 than 2019 or 2022 but that the migration was earlier in 2022.

Table 2 compares 2019, 2021 and 2021 with regard to the timing of the migration of willow-carrot aphids at Wellesbourne. Generally, the day-degree forecast gave useful information about the timing of activity in each year and the rankings between years were consistent.

Table 2. Comparisons between 2019, 2021 and 2022 regarding the timing of the migration of willow-carrot aphids at Wellesbourne. Rankings are shown in brackets: (1) = earliest of the 3 years.

	2019	**2021**	**2022**
Forecast start of migration	23 April (2)	9 May (3)	20 April (1)
Date by which first aphid captured in suction trap (weekly samples)	28 April (2)	16 May (3)	17 April (1)

Date by which first aphid captured in water traps (weekly samples)	7 May (2)	18 May (3)	Before 10 May (1)
Forecast 10% migration	16 May (2)	29 May (3)	8 May (1)

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 of limited use for forecast validation (there are no dates with zero captures).

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 2019-2022 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.

In 2021, the aphid forecast was developed by AHDB into a forecasting tool that was hosted on the AHDB Horticulture web site and was available in 2021 and 2022.

Financial Benefits

In year 3 of the project the focus of field work has been on a control trial to look at optimising control strategies through a replicated block trial based at Warwick crop centre. Although these data suggest that current treatment programmes will reduce the impact of virus infections on carrot yield, the initial treatment and intensity of treatment will influence the degree of impact from virus in crops. Impacts have not been quantified due to the limited scope of the trial.

Action Points

- Early treatment may mitigate against impacts of virus, whilst not preventing virus infection over the season.
- The willow-carrot aphid appears to be the primary vector of carrot red leaf virus in the trials carried out within this project. The day-degree forecast should be used as a guide for initiating aphid management.

- In some seasons late season infection may be driven by the parsnip aphid. However, the impact of these late season infections is not known.

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 & 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.

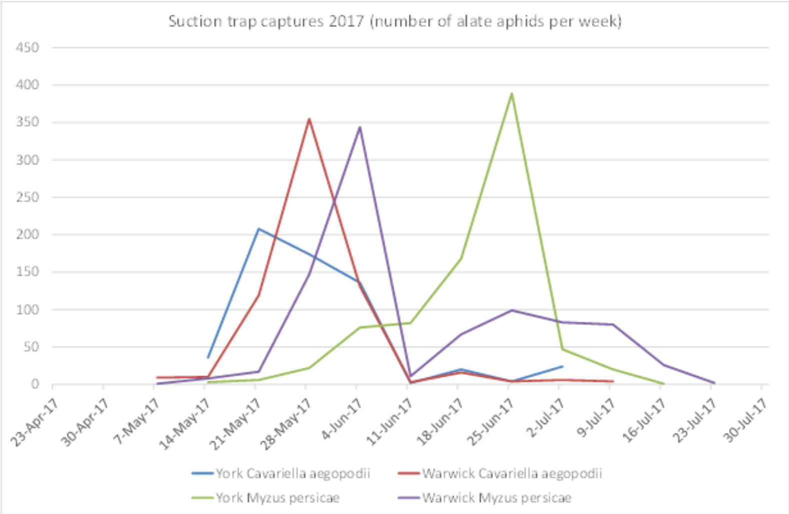

Figure 1. Numbers of alate aphids captured per week in suction traps at Fera (York) and Warwick Crop Centre (Wellesbourne, Warwick) in 2017 (data provided by the Rothamsted Insect Survey).

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 has looked at the presence of carrot infecting viruses in weed hosts, to try to ascertain the sources of carrot virus epidemics.

In the final year of the project the focus switched to efficacy of aphid control programmes with a 10 programme trial being conducted at Warwick University.

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 in 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 across three carrot beds. (See Figure 1.1) with ten (10) uncovering treatments and two (2) control plots (one covered all the time and one uncovered all the time). Each plot was 5m long with a 1m gap between plots. The first uncovered plot was from the initiation of the trial on the 23 April 2019, with each "weekly uncovered" plot exposed to aphids and virus pressure on successive weeks in the order shown in figure 1.1. Two yellow water traps were placed in the plots and these were reset weekly. One yellow water trap was situated in the uncovered control for the duration of the trial, the other trap was located in the weekly uncovered plot. At the end of each week the exposed sections were re-covered and further sections exposed.

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 captures were as described in 2019.

There were changes to the plot layout due to the randomised plot nature of the trial as shown in the Figure 1.4 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 1.4. 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 1.5. Of the aphids that are known to infest carrot, willow-carrot aphid (*C. aegopodii*) was the most abundant.

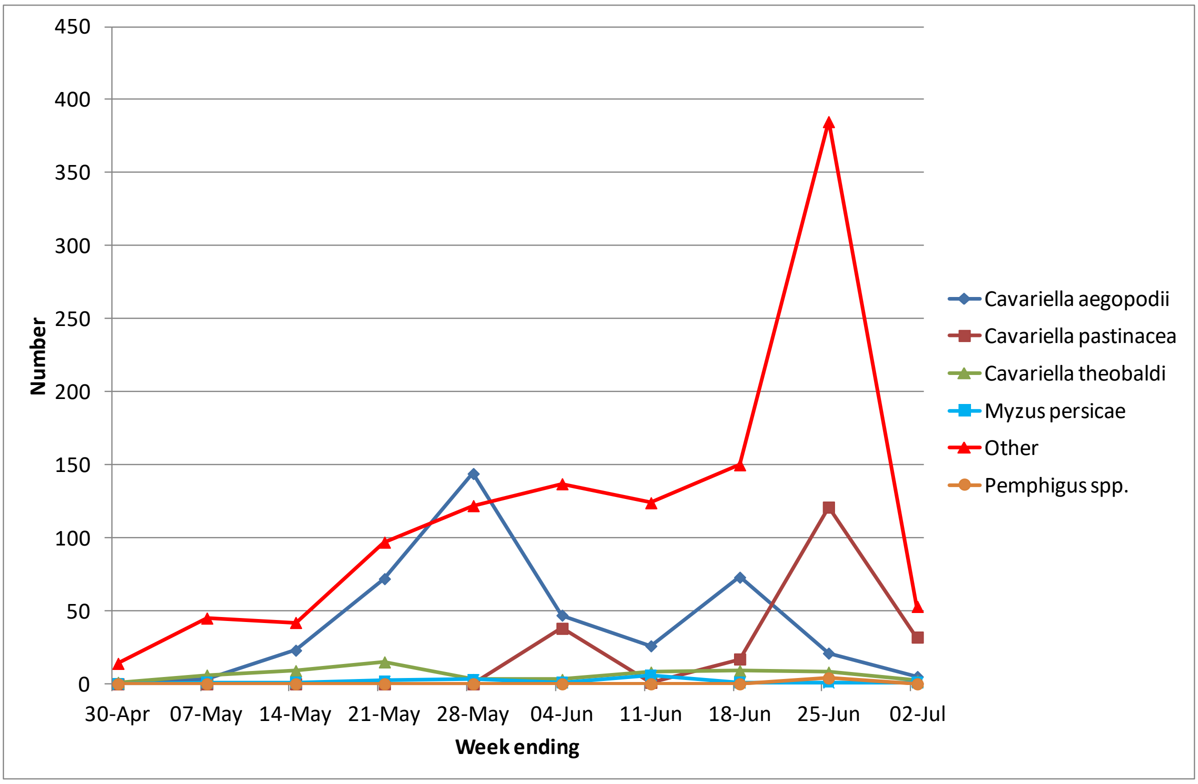

Figure 1.5. Total numbers of aphids captured per week in 4 water traps at Wellesbourne, Warwick in 2019. *C. pastinacea* and *C. theobaldii* are parsnip aphids.

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 1.6 (Stamford Bridge data) and Figure 1.7 (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.

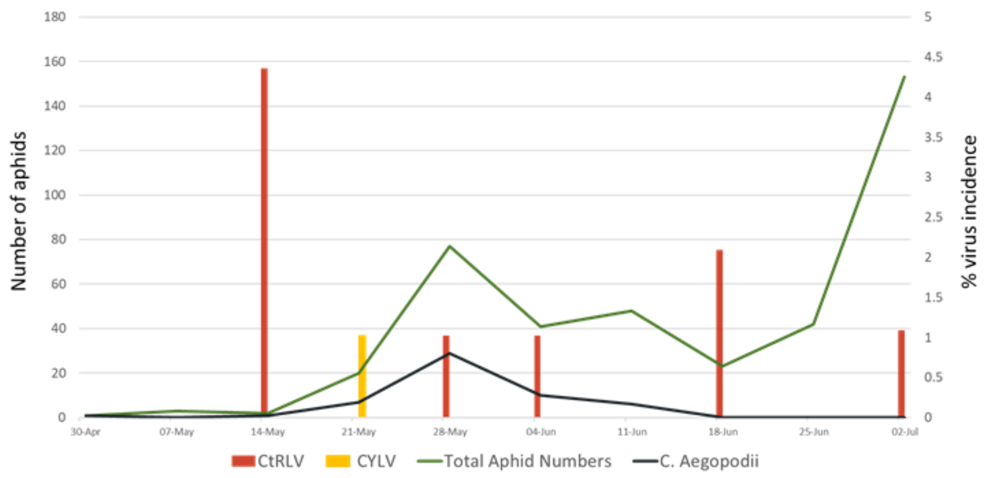

Figure 1.6. Weekly virus incidence and aphid numbers (Yellow water trap data) at Stamford Bridge, North Yorkshire. Virus data presented as % of individual viruses due to low incidence of virus transmission.

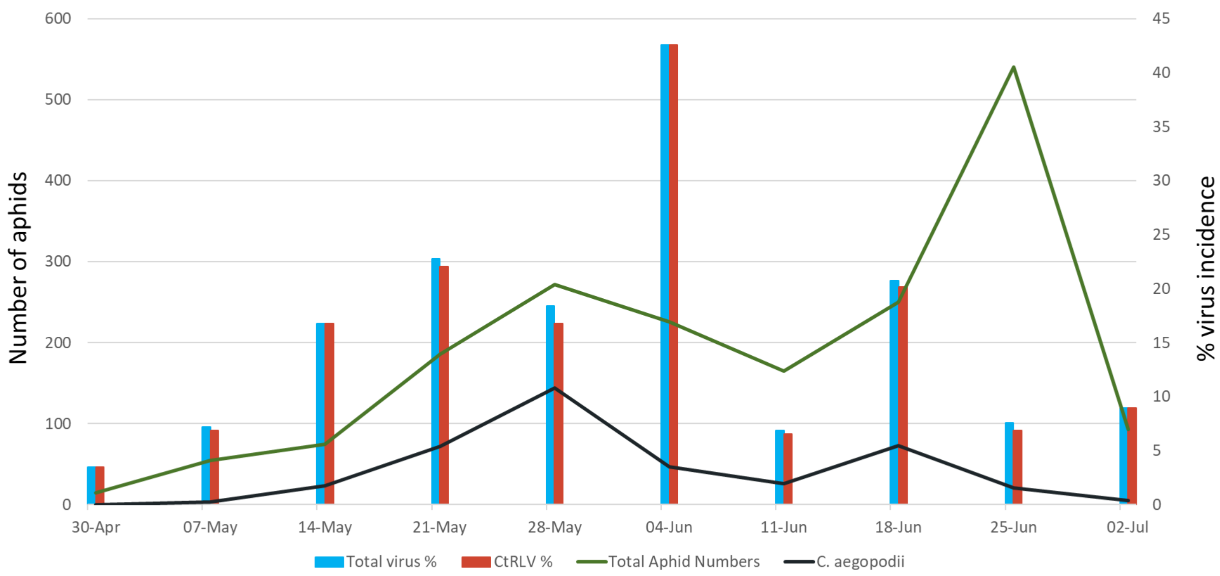

Figure 1.7. Weekly virus incidence and aphid numbers (yellow water trap data) at University of Warwick, Warwickshire. Virus data presented as % total virus and % carrot red leaf virus. Results are presented collated across all replicated plots.

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 captures 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 1.8 shows the mean yield in kg per plot. Figure 1.9 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.

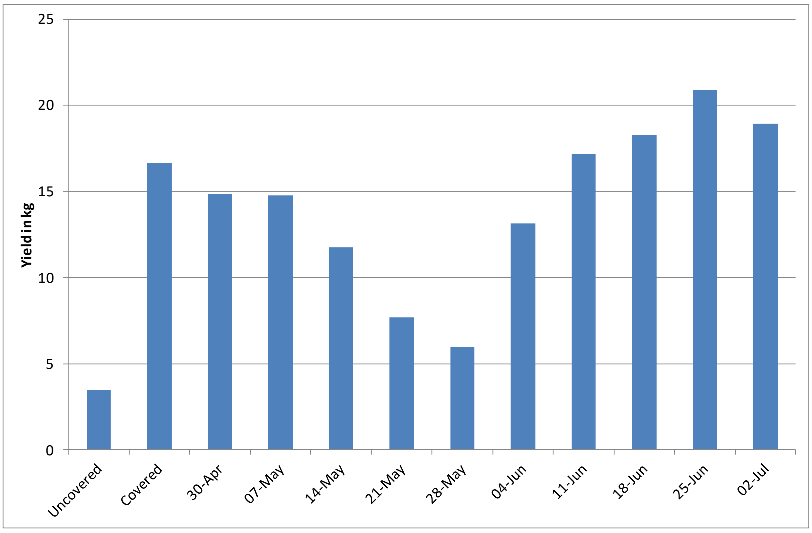

Figure 1.8. Yield in kg of carrots per plot (sample size 1.3m x 4 rows) on 19-20 November 2019.

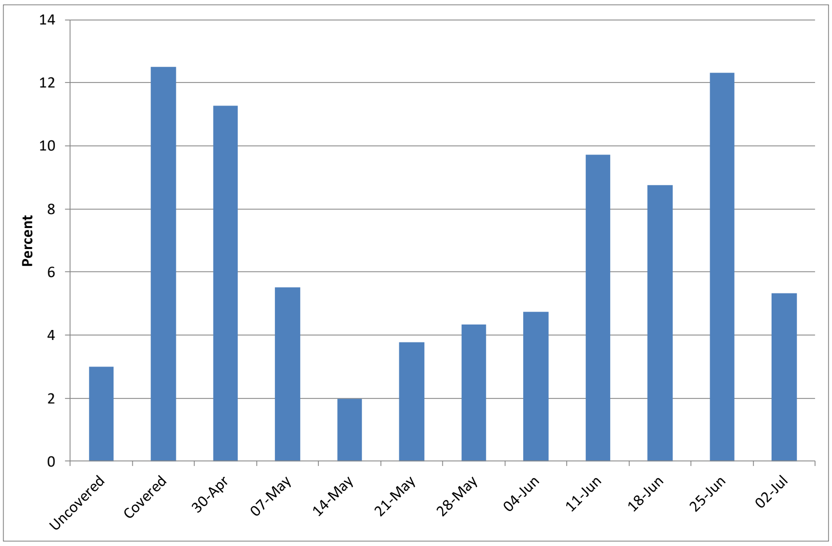

Figure 1.9. Mean percentage carrot roots undamaged by carrot fly (less than 5% of root surface damaged) (sample size 100 roots) rows) on 19-20 November 2019.

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 1.10) 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*.

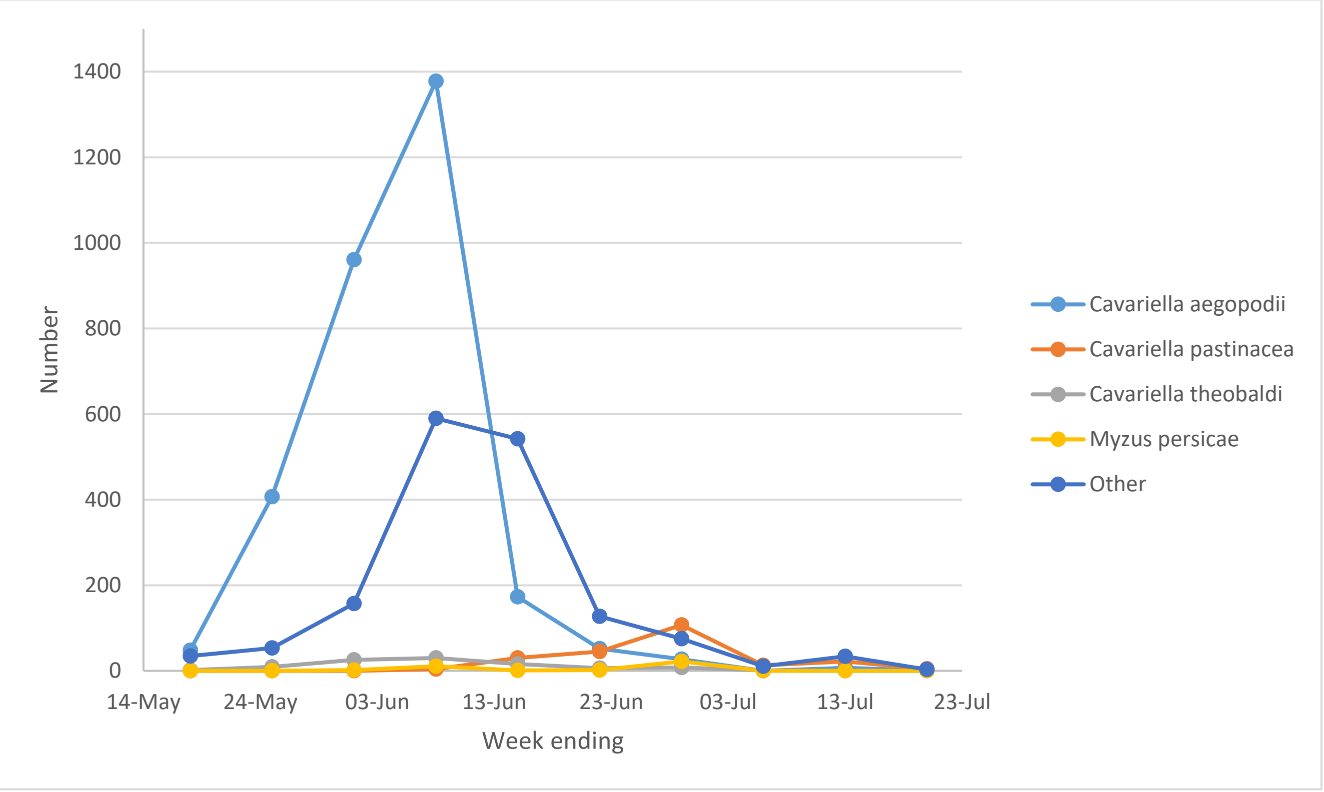

Figure 1.10. Timing of aphid captures in Warwick trial, all traps combined

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 1.11 (Fera-Buttercrambe data) and Figure 1.12 (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.

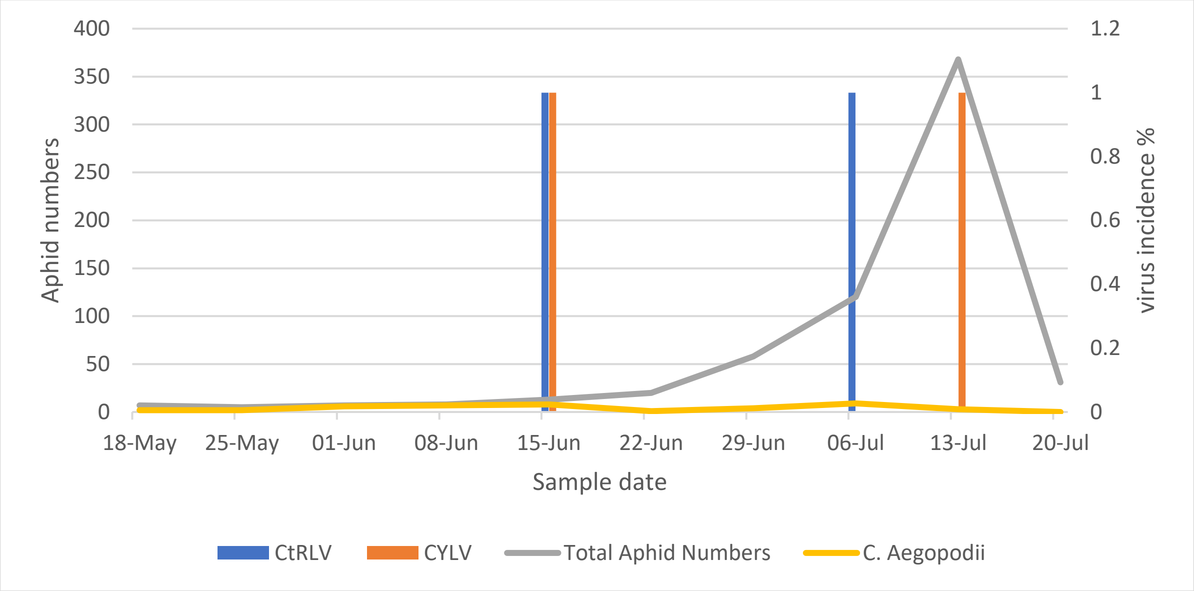

Figure 1.11. Incidence of carrot red leaf virus and carrot yellow leaf virus and aphid captures in the 2021 Fera-Buttercrambe trial.

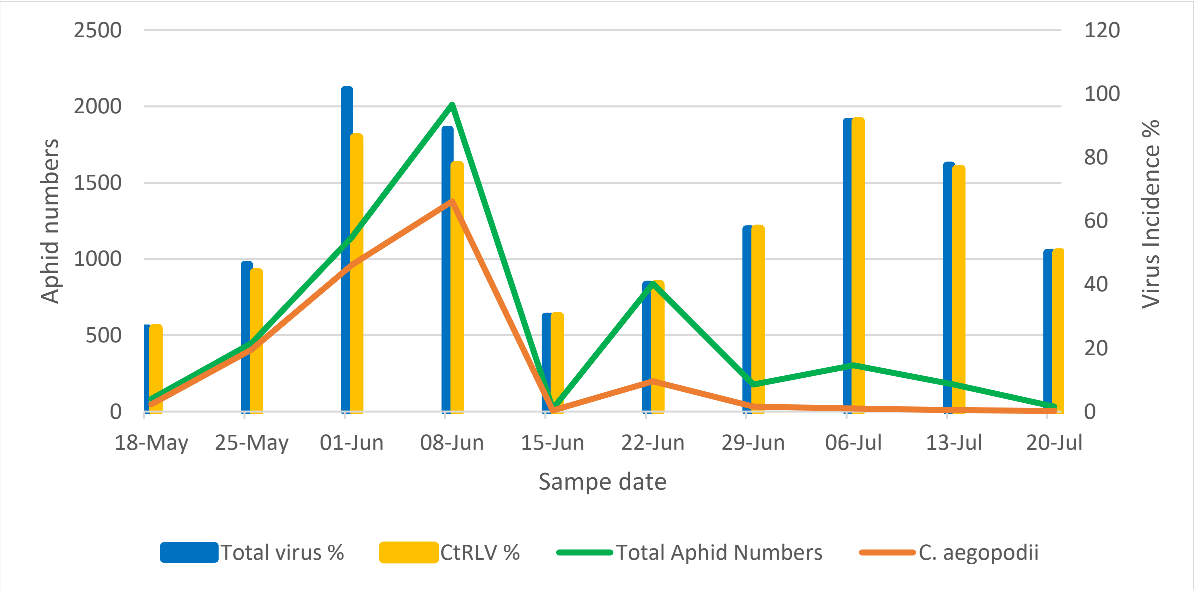

Figure 1.12. Incidence carrot red leaf virus and carrot yellow leaf virus and aphid captures in the 2021 Warwick trial.

Aphid captures and recorded virus transmission at the Warwick trial site are shown in Figure 1.12. 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 weeds.

	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.

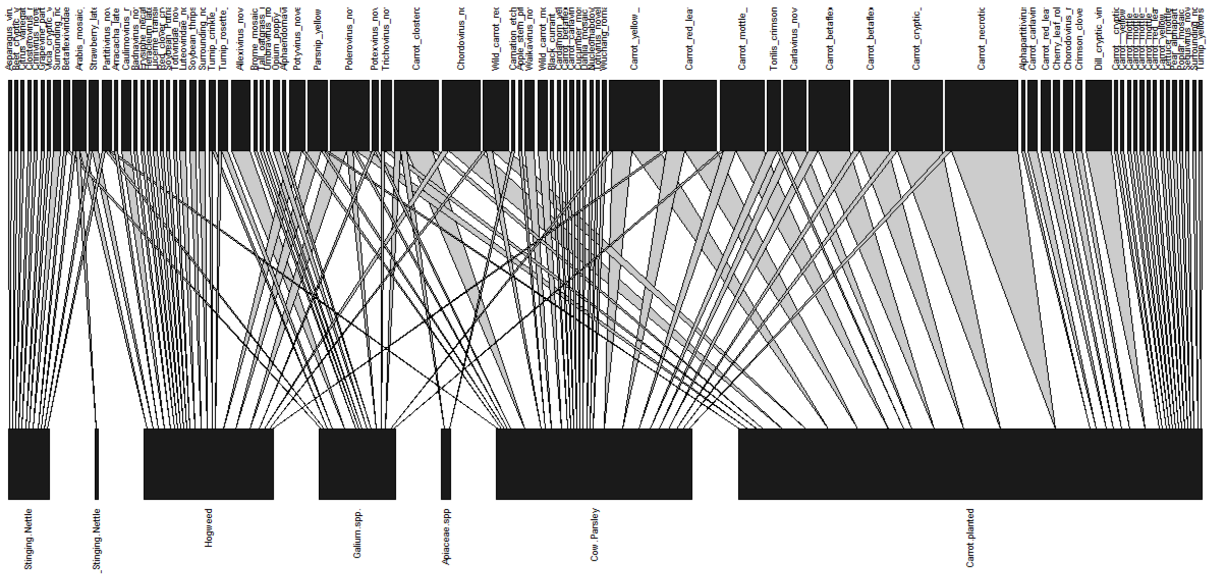

Figure 2.1. Network diagram composed of virus sequence data generated from carrot fields and associated weed samples, showing associations between known and novel viruses and the hosts in which they were detected.

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 traps)

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, nt = data not collected)

Date	**Numbers of aphids and ladybird larvae**							
2019	**Winged**		**Wingless**		**Parasitised aphids**		**Ladybird larvae**	
	LMC	**LMW**	**LMC**	**LMW**	**LMC**	**LMW**	**LMC**	**LMW**
25-Apr	0	0	0	0	0	0	nt	nt
01-May	0	0	0	0	0	0	nt	nt
09-May	0	2	3	8	0	0	nt	nt
14-May	4	11	1	4	0	0	nt	nt
22-May	22	23	8	14	2	1	nt	nt
30-May	25	16	27	47	2	5	1	0
06-Jun	21	4	391	344	2	6	nt	nt
13-Jun	3	10	133	266	2	7	3	6
20-Jun	2	5	162	221	2	10	nt	nt
27-Jun	1	2	95	143	1	9	nt	nt
04-Jul	1	2	17	18	1	1	nt	nt
11-Jul	1	0	2	22	1	0	nt	nt
17-Jul	1	0	5	12	0	1	nt	nt
24-Jul	0	0	4	1	0	0	nt	nt
06-Aug	0	0	0	0	0	0	nt	nt
20-Aug	0	0	0	0	0	0	nt	nt
04-Sep	1	1	21	10	0	0	nt	nt
19-Sep	1	0	42	6	0	0	nt	nt
03-Oct	1	nt	38	nt	0	nt	nt	nt

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.

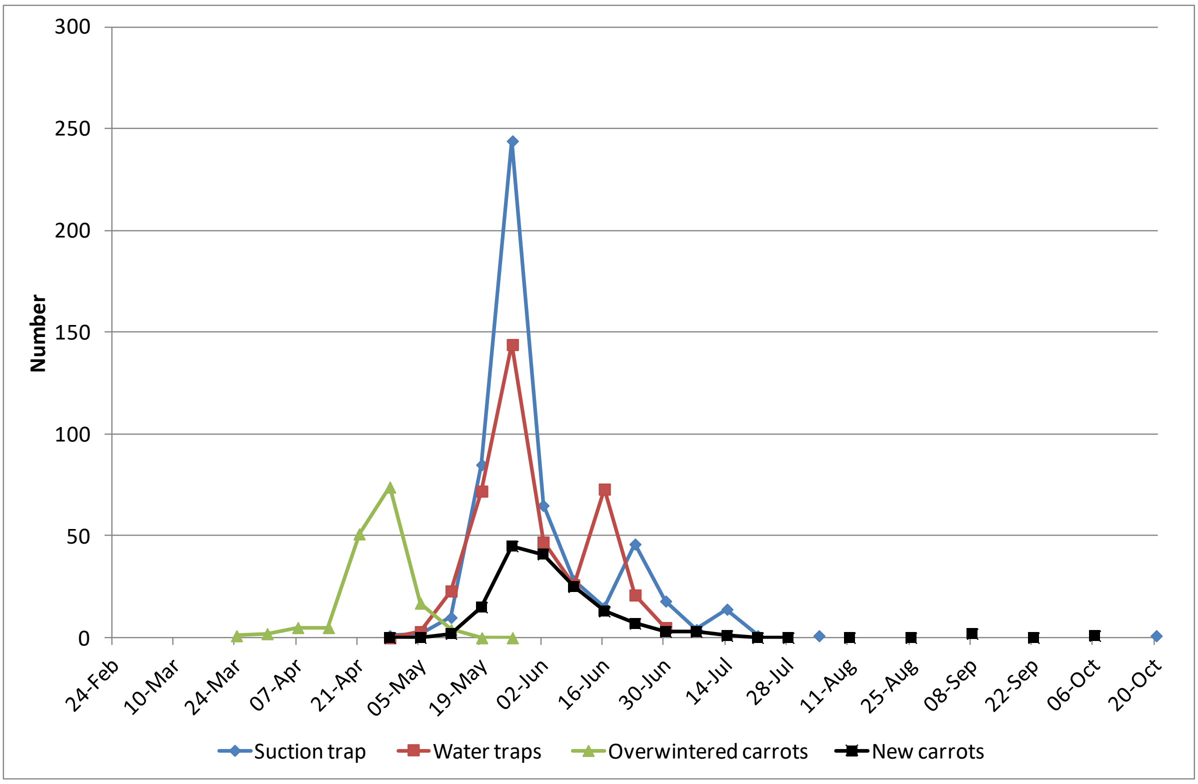

Figure 3.1. Numbers of winged willow-carrot aphid (C. *aegopodii)* captured in the suction trap, in the 4 yellow water traps in the field trial, on the plot of overwintered carrots and on plots of new carrots at Wellesbourne, Warwick in 2019.

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

26-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

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

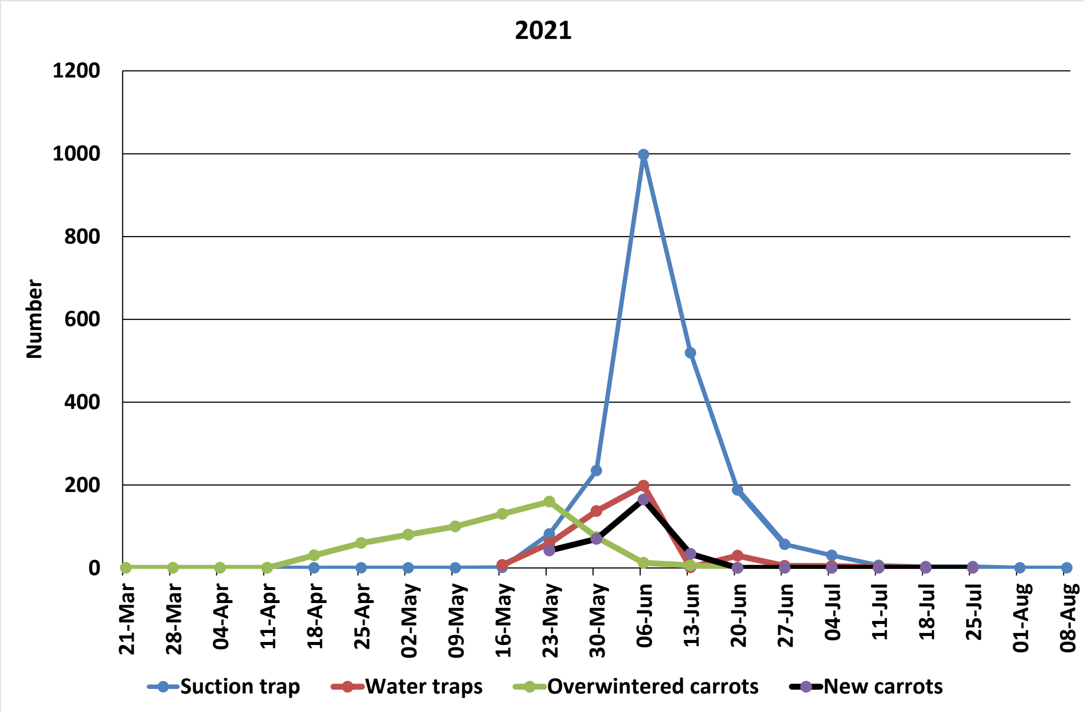

Figure 3.2. Numbers of winged willow-carrot aphid (C. *aegopodii)* captured in the suction trap, in the 4 yellow water traps in the field trial, on the plot of overwintered carrots and on plots of new carrots at Wellesbourne, Warwick in 2021.

Field data 2022

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 2021-22. Table 3.7 shows the numbers of aphids on the overwintered carrots from January 2022. Aphids were present from before the beginning of 2022 and numbers of winged and wingless aphids peaked in late April to mid-May. The numbers of parasitised aphids (aphid mummies) were also recorded.

Table 3.7. Numbers of aphids per plant on 3 x 0.5 m lengths of row of overwintered carrots at Wellesbourne in 2022.

	Winged	Wingless	Parasitised
5-Jan-22	0.02	1.10	0.00
24-Jan-22	0.00	2.03	0.00
15-Feb-22	0.00	1.78	0.00
8-Mar-22	0.00	1.89	0.00
23-Mar-22	0.03	30.78	0.00
7-Apr-22	0.50	57.27	0.00

21-Apr-22	4.83	96.67	0.00
10-May-22	2.38	117.79	0.04
24-May-22	0.02	3.36	0.00

Aphids were also monitored on the new carrots sown in late March 2022 (Table 3.8). Winged aphids had arrived in the plots by 10th 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 mid-October. A small number of aphids were parasitised in May - June.

Table 3.8. Numbers of willow-carrot aphid per plant on 3 x 0.5 m lengths of row of newly-sown carrots (March) at Wellesbourne in 2022.

	Winged	**Wingless**	**Parasitised**
10-May-22	0.07	0.09	0.00
17-May-22	0.09	0.14	0.00
24-May-22	0.12	1.46	0.00
30-May-22	0.08	1.78	0.02
7-Jun-22	0.02	3.23	0.00
13-Jun-22	0.01	1.93	0.01
21-Jun-22	0.01	1.74	0.00
27-Jun-22	0.00	1.61	0.00
7-Jul-22	0.00	0.82	0.00
12-Jul-22	0.01	0.59	0.00
19-Jul-22	0.00	0.00	0.00
25-Jul-22	0.00	0.04	0.00
1-Aug-22	0.00	0.00	0.00
10-Aug-22	0.00	0.00	0.00
24-Aug-22	0.00	0.00	0.00
12-Sep-22	0.00	0.00	0.00
19-Oct-22	0.01	0.40	0.00

Summary data for willow-carrot aphid from the network of Rothamsted Suction traps are shown in Table 3.9. The numbers of willow-carrot aphid at Wellesbourne peaked in the week ending 15 May.

Table 3.9. Summary of captures of willow-carrot aphid in 2022 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																	
10-Apr					0	0	0	0	0	0		0	0	0	0	0	**0**
17-Apr		0	1		2	0	0	5	0	1		0	0	1	3	1	**14**
24-Apr	0	0	0		0	1	1	0	1	1		1	0	0	4	1	**10**
01-May	0	0	0	0	0	0	0	14	27	1		4	3	3	15	0	**67**
08-May	0	0	0	0	0	2	11	52	101	18	3	14	25	11	60	9	**306**
15-May	0	1	1	0	0	18	2	28	111	30	2	41	73	7	67	39	**420**
22-May		2	3		5	94	12	160	418	17		35	176	7	60	46	**1035**
29-May					0	6	5	21	59	6	2	8	30	1	14	9	**161**
05-Jun					2	12		24	37		0	11	7	2	9	9	**113**
12-Jun		1	1		0	2	3	15	81	8	0	11	17	2	4	4	**149**
19-Jun	0	2	5		4	12	2	12	21	7	3	5	5	1	4	4	**87**
26-Jun	0	0	6		15	13	3	9	8	6	0	1	0	0	1	4	**66**
03-Jul	1	5	1		7	0	3	2	1		0	0	1	1	1	0	**23**
10-Jul	0	5	2		0	5	1	2	2	0	0	0	12	1	0	2	**32**
17-Jul	0	1	2	1	2	0	0	1	5	0	0	1	0	0	0	0	**13**
24-Jul		2	1		0	0	1	0	0	0	1	0	0	0	0	0	**5**
31-Jul		0	0		0	0	0	0	0	0	0	0	0	0	0	1	**1**
07-Aug			0		0	0	0	0	0	0	0	0	0	0	0	0	**0**
14-Aug		0	0		0	0	1	0	0	0	1	0	0	0	0	0	**2**
21-Aug	3	0	0		0	0	0	0	0	0	0	0	0	0	0	0	**3**
28-Aug			1		1	0	0	0	0	0	0	0	0	0	0	0	**2**
04-Sep			0		0	0	0	0	0	0	0	0		0	0	0	**0**
11-Sep					1	3	0	0	0	1	0	0	0	0	0	0	**5**
18-Sep		23	1		0	1	0	0	1	0	1	0	0	0	0	1	**28**
25-Sep					0	5	3	0	2	1	1	0	1	1	0	0	**14**
02-Oct					0	14	0	0	8		2	0	0	0	0	0	**24**
09-Oct					0	27	8	28	19	0	1	0	0	0	0	0	**83**
16-Oct					0	116	29	14	8	7	0	0	0	0	0	1	**175**
23-Oct					2	1357	57	8	1	0	0	0	0		0	0	**1425**
30-Oct					1	338	30	0	1	0	1	0	0	0	1	1	**373**

Figure 3.3 compares the pattern of captures of willow-carrot aphid in the suction trap and the water traps at Wellesbourne in 2022 with the numbers of winged aphids found on carrot plants (Tables 3.7 & 3.8). 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 and 2021, winged aphids were present on the overwintered carrots well before they were captured in traps.

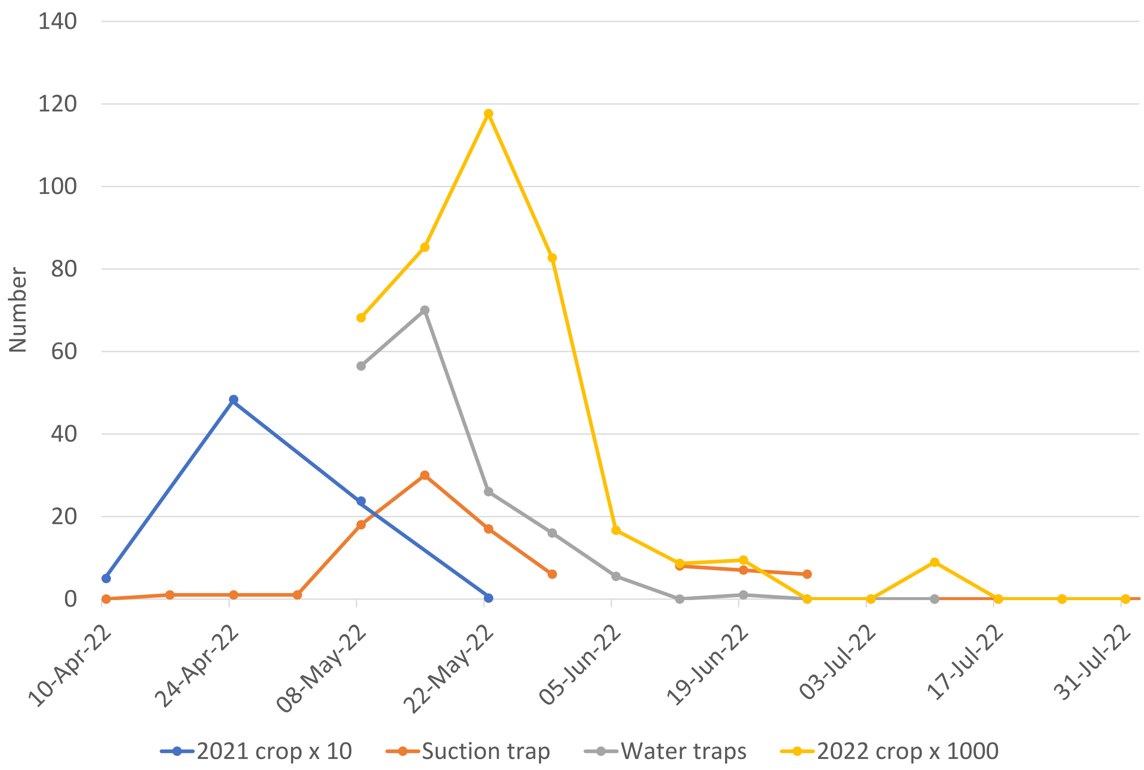

Figure 3.3. Numbers of winged willow-carrot aphid (C. *aegopodii)* captured in the suction trap, in the 4 yellow water traps in the field trial, on the plot of overwintered carrots and on plots of new carrots at Wellesbourne, Warwick in 2022.

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 1 January or 1 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 4.4ºC 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.

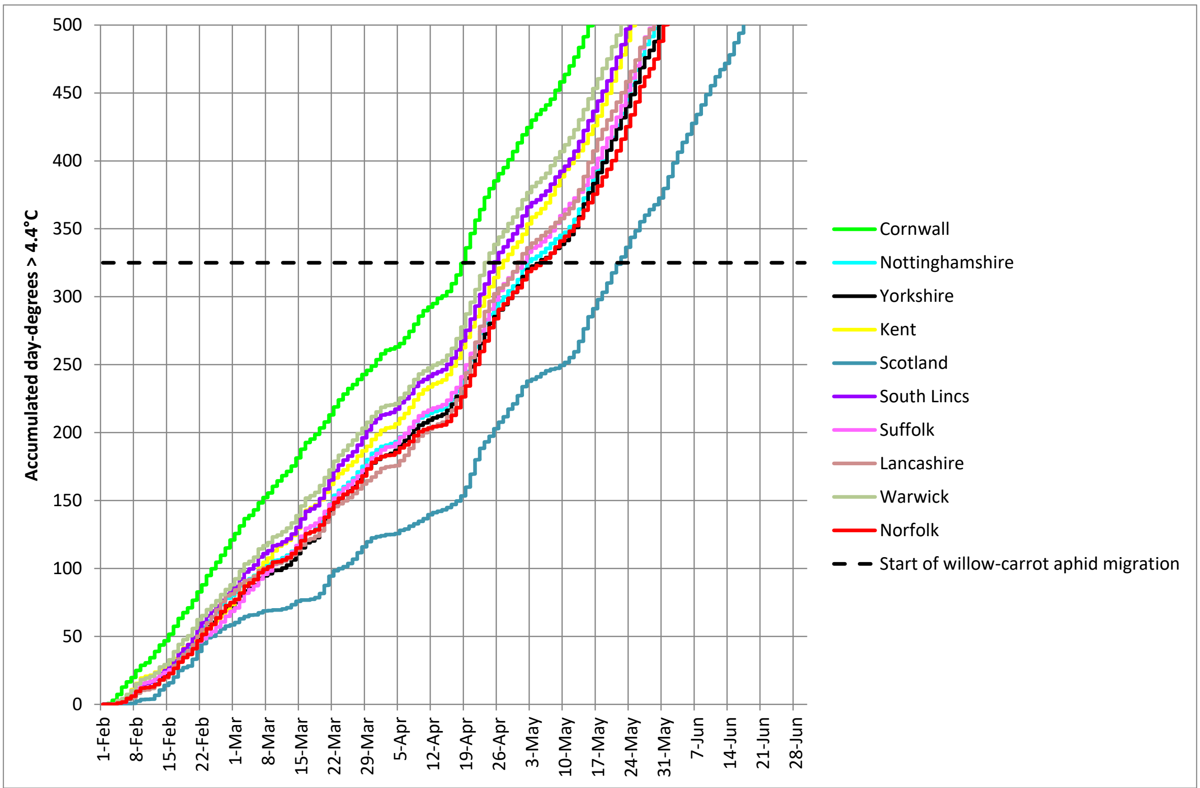

Figure 3.4. Day-degree forecasts for 2019 for the start of the willow-carrot aphid 'migration' to susceptible crops. The migration is forecasted to begin when 325 day-degrees above 4.4ºC have been accumulated from 1 February.

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 4.4ºC 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.

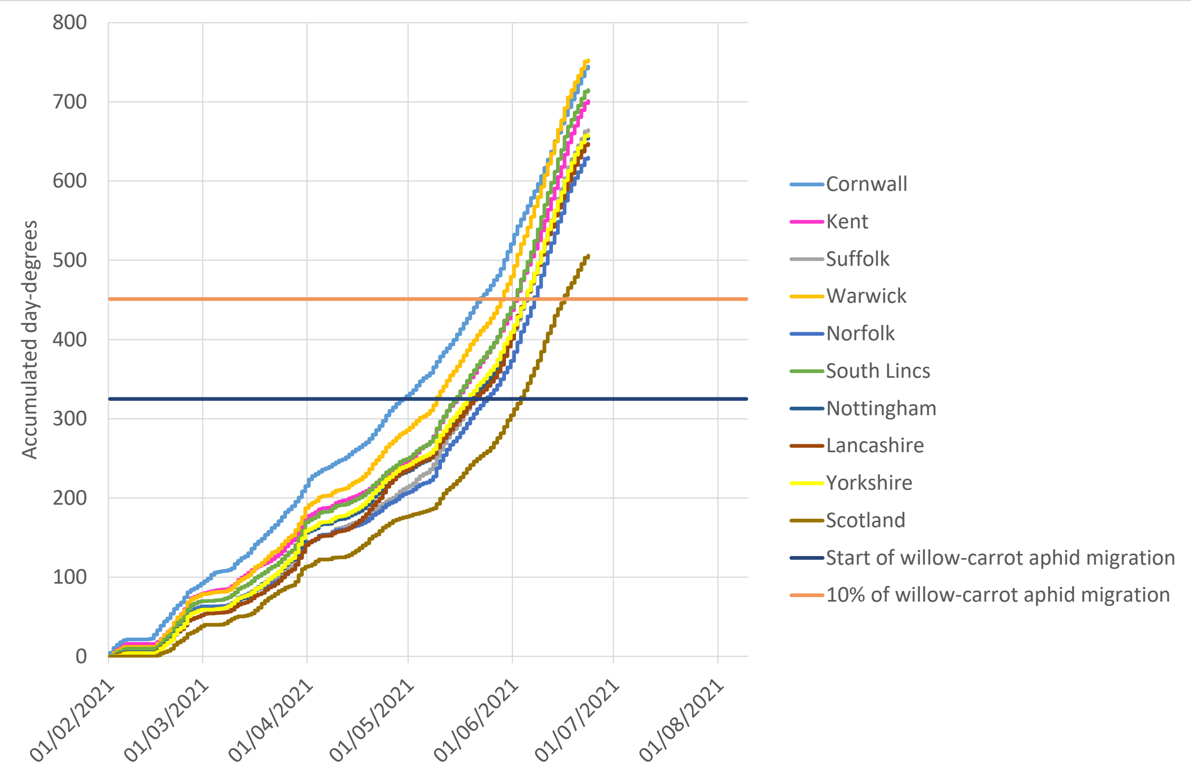

Figure 3.5. Day-degree forecasts for willow-carrot aphid in 2021. Information from the Rothamsted Suction trap captures have been used to estimate the mean number of D° from 1 February until the first aphid of the year is caught in a suction trap (the start of the migration to carrot) and when 10% of aphids are caught. This is after approximately 325 and 451D° respectively.

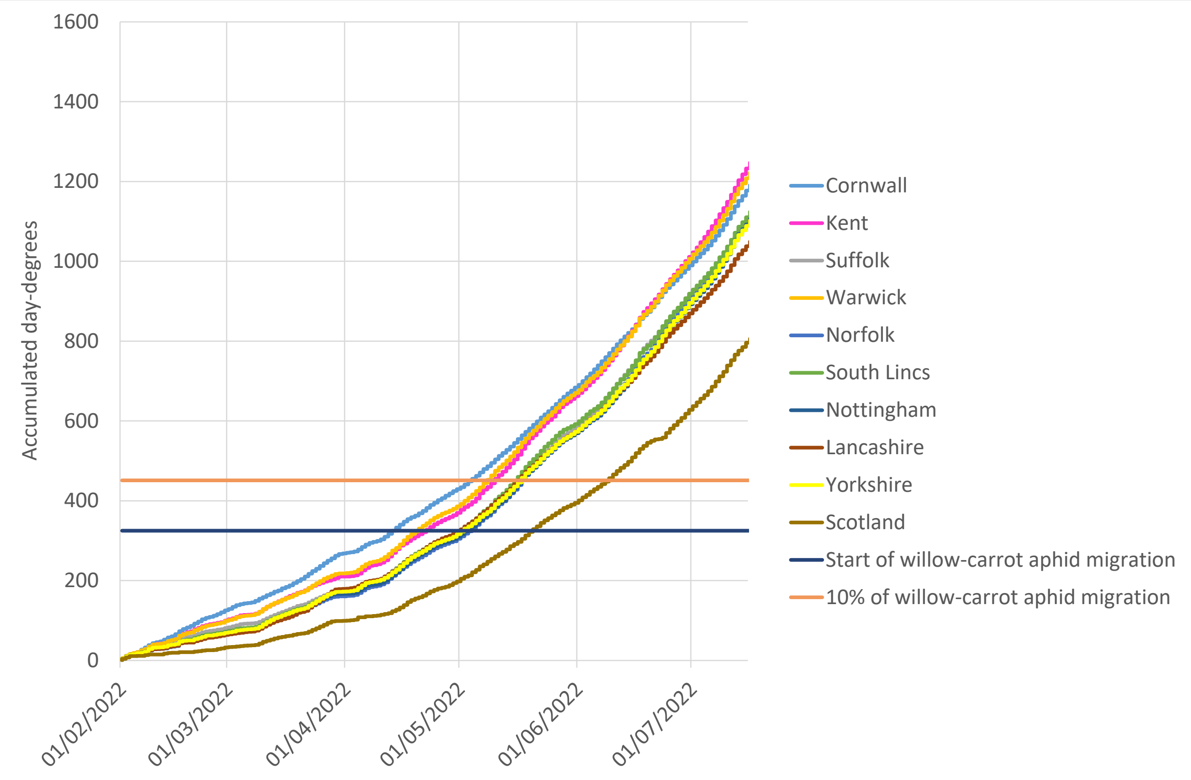

Figure 3.6. Day-degree forecasts for willow-carrot aphid in 2022. Information from the Rothamsted Suction trap captures have been used to estimate the mean number of D° from 1 February until the first aphid of the year is caught in a suction trap (the start of the migration to carrot) and when 10% of aphids are caught. This is after approximately 325 and 451D° respectively.

3.4 Comparisons between years

Figure 3.7 compares suction trap captures at Wellesbourne in 2019, 2021 and 2022, confirming that willow-carrot aphids were most abundant in 2021 but that the migration was earliest in 2022.

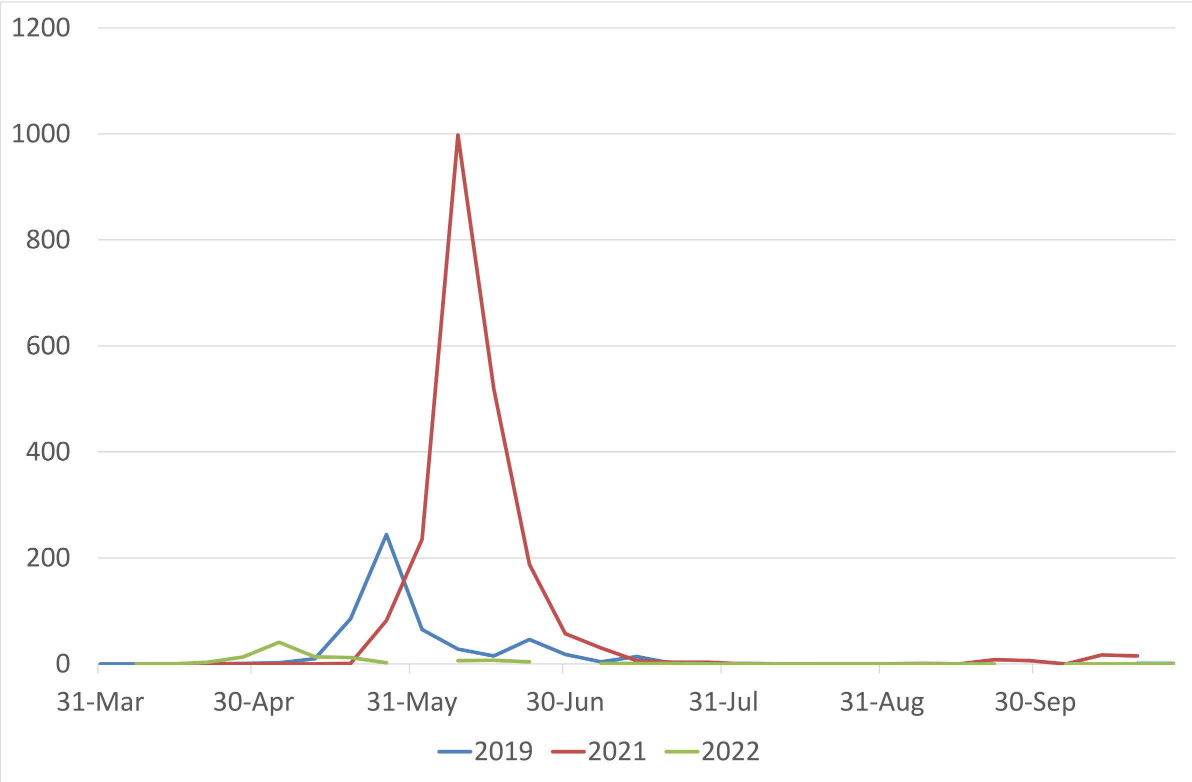

Figure 3.7. Suction tap captures at Wellesbourne in 2019, 2021 and 2022 confirming that willow-carrot aphids were more abundant in 2021 than 2019 or 2022 but that the migration was earlier in 2022.

Table 3.1 compares 2019, 2021 and 2021 with regard to the timing of the migration of willow-carrot aphids at Wellesbourne. Generally, the day-degree forecast gave useful information about the timing of activity in each year and the rankings between years were consistent.

Table 3.1. Comparisons between 2019, 2021 and 2022 regarding the timing of the migration of willow-carrot aphids at Wellesbourne. Rankings are shown in brackets: (1) = earliest of the 3 years.

	2019	**2021**	**2022**
Forecast start of migration	23 April (2)	9 May (3)	20 April (1)
Date by which first aphid captured in suction trap (weekly samples)	28 April (2)	16 May (3)	17 April (1)

Date by which first aphid captured in water traps (weekly samples)	7 May (2)	18 May (3)	Before 10 May (1)
Forecast 10% migration	16 May (2)	29 May (3)	8 May (1)

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 3.7-3.9 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 captures but for Grampian the water trap captures seem later than the captures in the suction trap at Dundee (the nearest functioning suction trap).

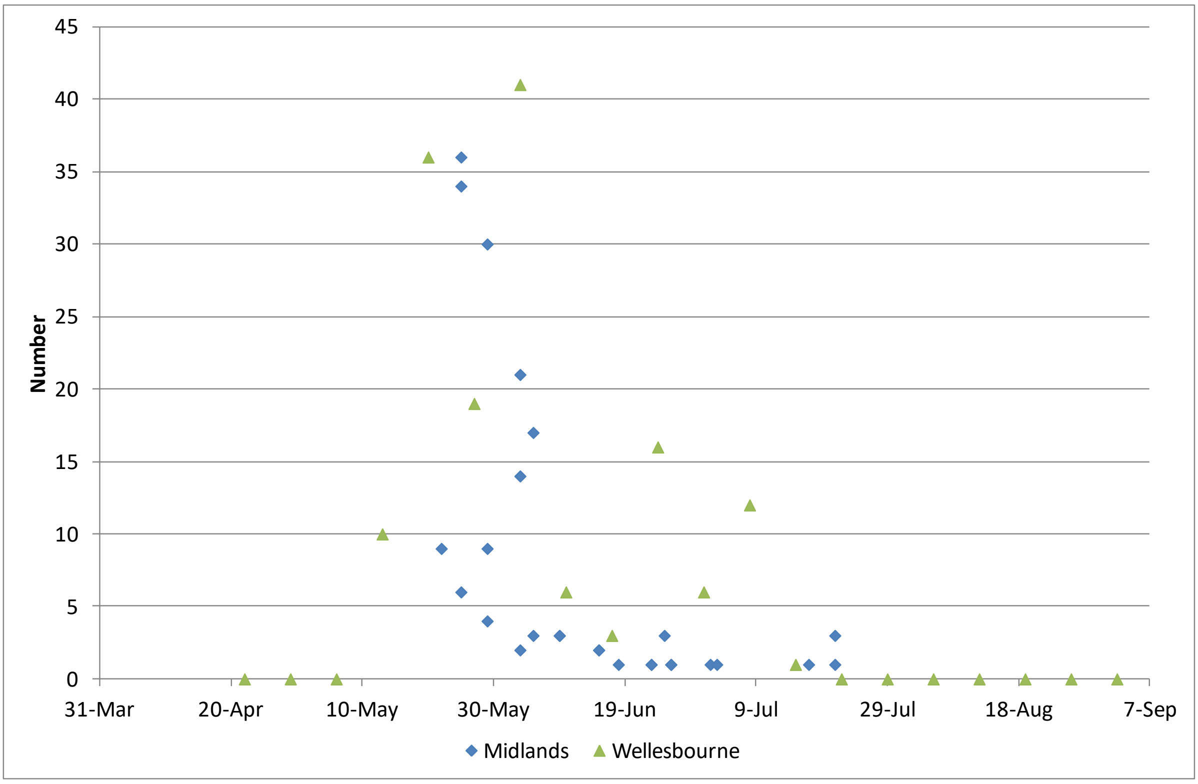

Figure 3.8. Scatter plot comparing data from Fera Science Ltd water trap samples in the Midlands region in 2018 with suction trap data from Wellesbourne. Vertical axis "number" = number of aphids.

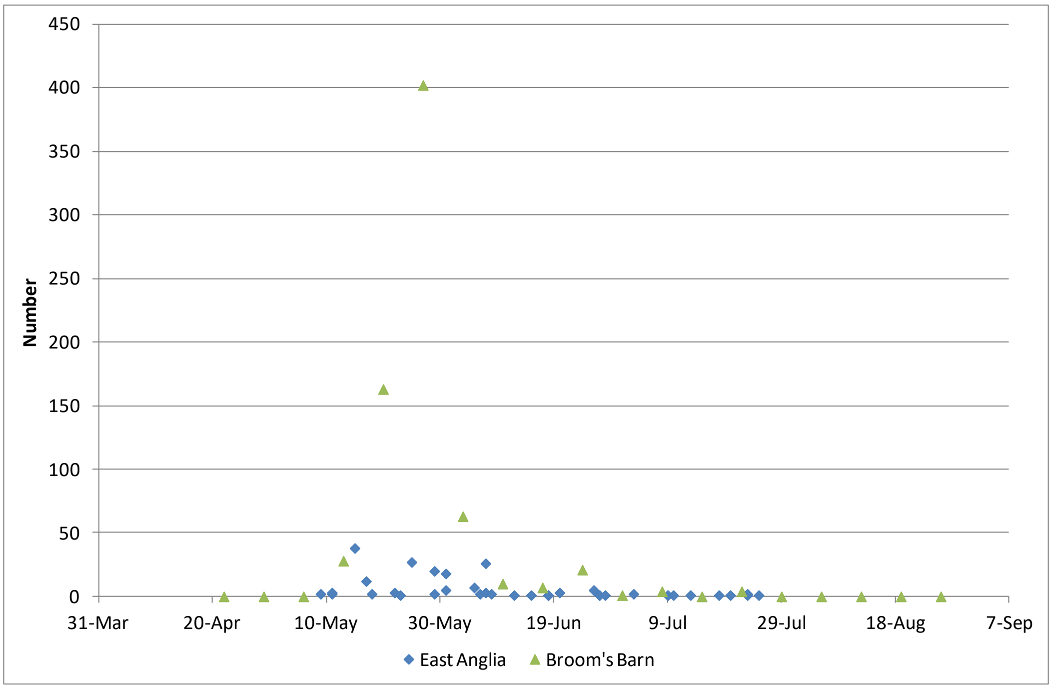

Figure 3.9. Scatter plot comparing data from Fera Science Ltd water trap samples in the East Anglia region in 2018 with suction trap data from Broom's Barn. Vertical axis "number" = number of aphids.

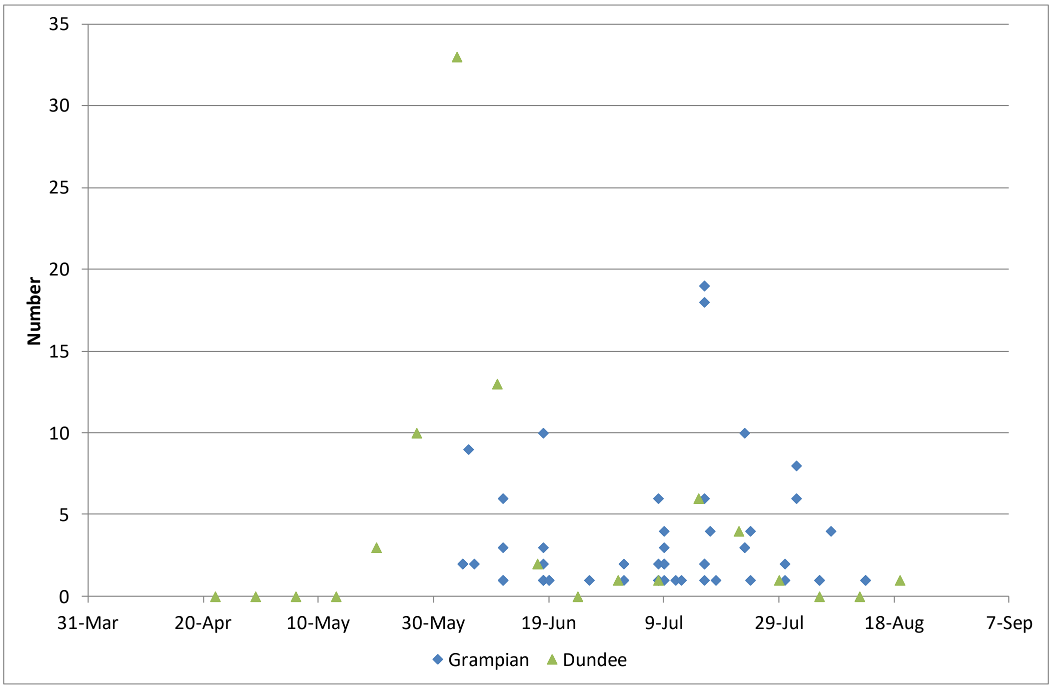

Figure 3.10. Scatter plot comparing data from Fera Science Ltd water trap samples in the Grampian region in 2018 with suction trap data from Dundee. Vertical axis "number" = number of aphids.

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 2019-2022 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 3.11 shows the summarised data for 2021.

In 2021, the aphid forecast was developed by AHDB into a forecasting tool that was hosted on the AHDB Horticulture web site.

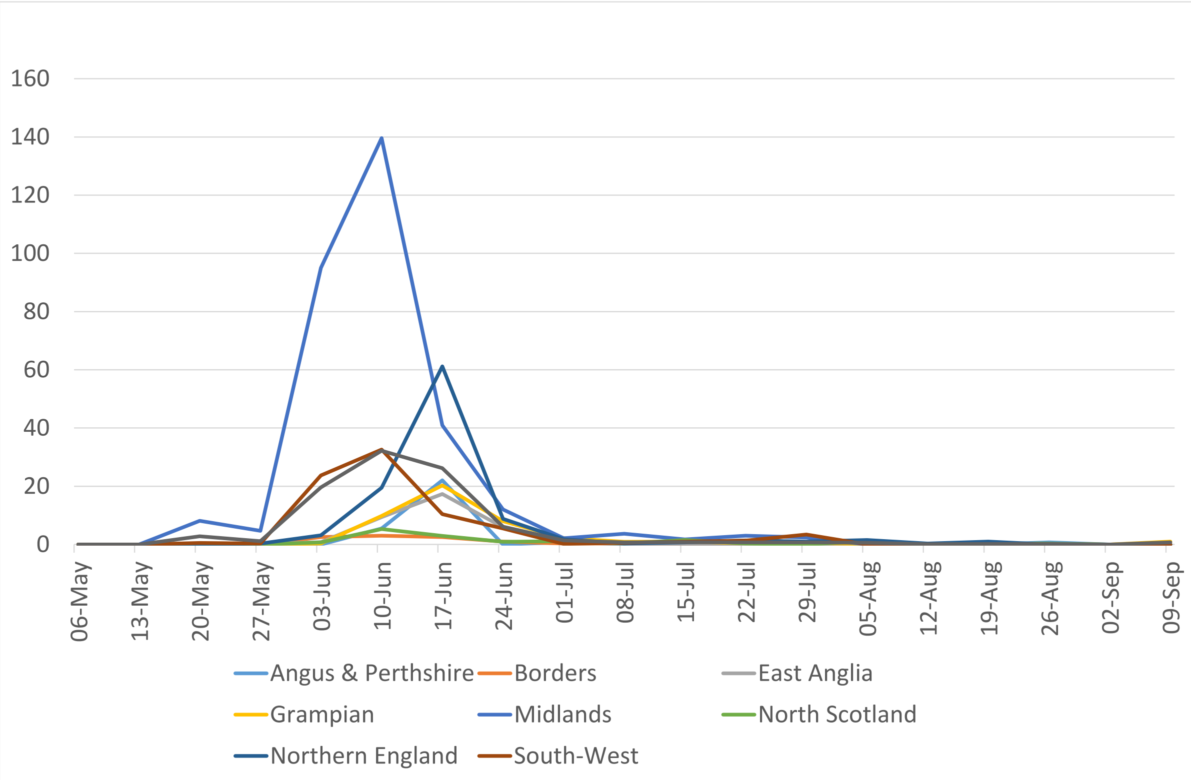

Figure 3.11. Data (numbers of aphids) summarised by region from the Fera/AHDB potato water trap data sets for 2021 that were made available to the AHDB Pest Bulletin on a weekly basis.

Objective 4. Spray control trial

4.1 Background

Following two years of comparative field work at Wellesbourne and in the York area, data had been gathered on the timing of flights of vector aphid species, indicating the majority of transmission into field plots occurred in the first half of the trial, associated with the movement of *Cavariella aegopodii*. In some seasons (e.g. 2022) some late season transmission may also be driven by other vectors such as *C. pastinaceae,* however the relative impacts of this late season transmission on yield and quality were unknown. Therefore, a management and control trial was planned for the 2023 growing season based in the trial field at Warwick University, Wellesbourne, Warwickshire. The aim of the trial was to investigate the efficacy of different control regimes with currently available products.

4.2 Materials and Methods

The trial was conducted over the course of ten weeks with a range of different treatment strategies. A total of twelve treatment plots, consisting of ten treatments in addition to positive (intensive conventional treatment) and negative (untreated) controls. The treatments applied to the plots were selected based on available products to reflect current options for control. In some cases, these were applied as single product, in others combination treatments were trialled. Additionally, some treatments selected would focus on the early part of the season, investigating intensive treatment to limit virus entry early season, whereas other treatments would investigate season long treatment options. These treatments are detailed in Table 4.1 below. The twelve treatment plots were replicated four times which gave a total of forty-eight plots. Treatments were arranged following a randomised block design.

All sampling and virus testing was carried out following the same bulk testing principles used in the two previous years whilst investigating the timing of transmission and vector associations with carrot red leaf virus and carrot yellow leaf virus. To allow for transmitted virus to bio-amplify in the infected plants there was a four-week lag between a treatment and the corresponding sampling week. Briefly, four weeks after the initial treatment, 20 sub-samples consisting of five leaves each were sampled from each untreated control plot on a weekly basis to give a measure of virus transmission into the trial throughout the season. This would be used to monitor the timing of virus entering the plots. At the mid-point and at the end of the trial samples were taken from all plots. Four weeks after the week 5 treatment and again after the week 10 treatment, 200 leaves sampled as 50 leaves per plot (40 x 5 leaf bulks) were sampled from all plants to give an indication of virus content across the different treatments. After sampling, all bulked leaf samples were sent to Fera for processing and testing for the presence of carrot red leaf virus (CtRLV) and carrot yellow leaf virus (CYLV) as per previous testing.

Table 4.1. Treatment plan for vetor control trial.

Treatment	T1	T2	T3	T4	T5	T6	T7	T8	T9
Timing [1]		7DAT1	7DAT2	7DAT3	7DAT4	7DAT5	7DAT6	7DAT7	7DAT8
Date	09-May	17-May	25-May	31-May	07-Jun	14-Jun	20-Jun	28-Jun	06-Jul
1	Untreated	Untreated	Untreated	Untreated	Untreated	Untreated	Untreated	Untreated	Untreated
2	Movento 0.3	Movento 0.3	Movento 0.3	Movento 0.3	Movento 0.3	Movento 0.3	Movento 0.3	Movento 0.3	Movento 0.3
3	Movento 0.3		Teppeki 0.14		Movento 0.3		Teppeki 0.14		
4	Teppeki 0.14		Movento 0.3		Teppeki 0.14		Movento 0.3		
5	Gazelle 0.2		Movento 0.3		Teppeki 0.14		Movento 0.3		Teppeki 0.14
6	Movento 0.3		Gazelle 0.2		Teppeki 0.14		Movento 0.3		Teppeki 0.14
7	Movento 0.3	Teppeki 0.14	Movento 0.3	Teppeki 0.14					
8	Movento 0.3		Movento 0.3						
9	Coded 0.25		Coded 0.25						
10	Teppeki 0.14		Teppeki 0.14						
11	Gazelle 0.2		Gazelle 0.2						
12 [2]	Minecto One		Minecto One						

3- 7DATX represents "days after treatment"
4- Minecto One - only carrot fly control is specified on the label.

To monitor the timing of aphid movements into the trial, four yellow water traps were placed in the untreated control plots. Aphids from these traps were processed at Fera where they were identified and counted in line with the procedures from the first two years of the project.

During the trial visual symptom expression was recorded (week 8, 29/06/2022). On completion of the trial carrot roots were assessed for yield (total weight in Kg from 1.5m of each of the middle two rows). Additionally, roots were assessed for visual symptoms of root tip necrosis, associated with infection by carrot yellow leaf virus, and also for symptoms of carrot fly damage.

4.3 Results

4.3.1 Aphid captures and monitoring of virus transmission

Aphids were caught using four yellow water pan traps in the untreated control plots (Figure 4.1). These data indicated that vector pressure from the key vector species, *Cavariella aegopodii* was high from the start of the trial, but migrations into the crop had finished by the week of 16 June (Treatment week 6). Numbers of both *C. aegopodii* and *Myzus persicae* peaked in the second week of the trial.

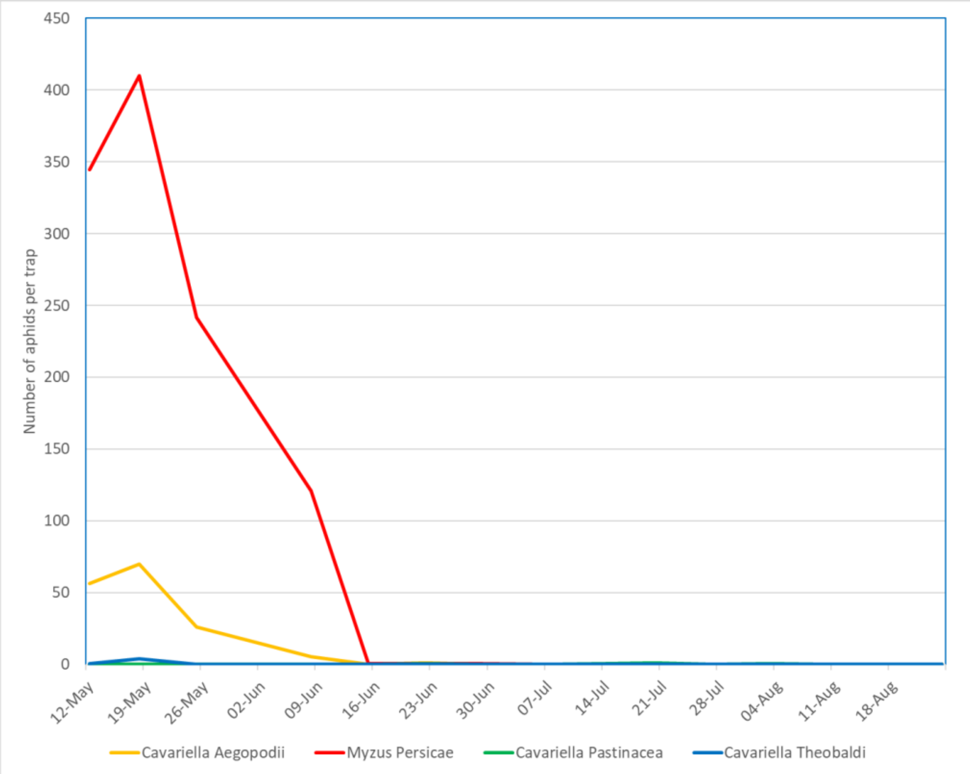

Figure 4.1. Number of aphids per trap caught in the four traps in the untreated control plots.

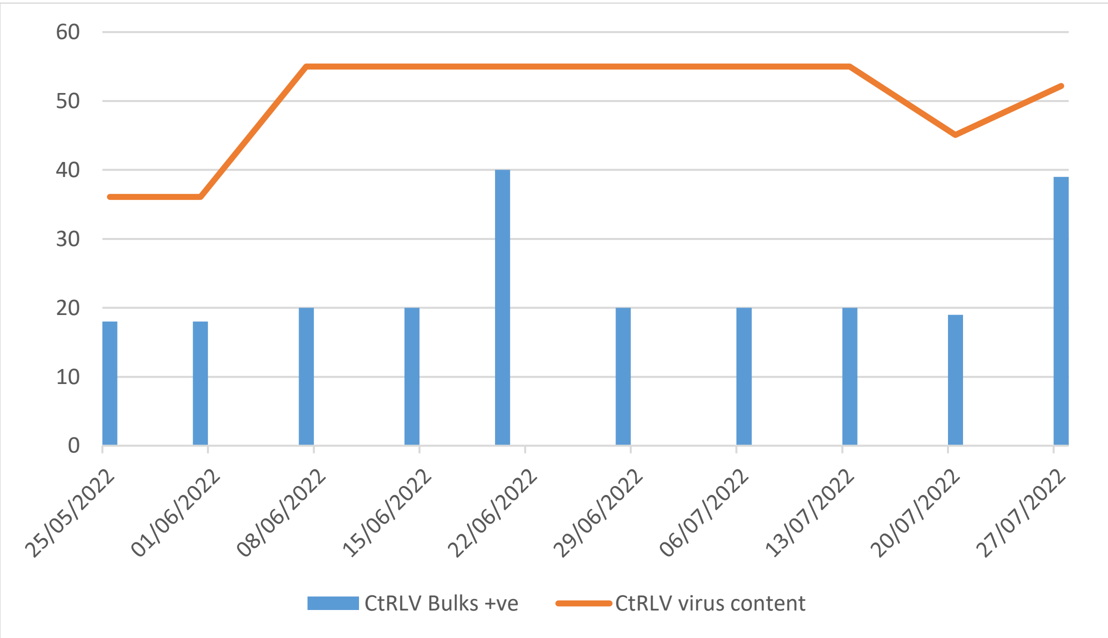

Figure 4.2. Number of bulk samples positive (blue bars) and calculated mean % virus content (orange line). Weeks 1-4 and 6-9 were sampled as 20 x 5 leaf bulks. Weeks 5 and 10 (22/06/22 and 27/07/22) were sampled as 40 x 5 leaf bulks.

The weekly virus testing of the uncovered control plots indicates that there was high transmission pressure from the very first week of the trial. Corresponding to the early movement of aphids into the field plots, virus was detected at high prevalence from the first week of the trial with 18 of the 20 bulked samples testing positive for Carrot red leaf virus, calculated as approximately 36% virus (calculated virus content 36%, CI: 20.53-58.47). By the third week of sampling all bulked subsamples tested positive for the virus. Due to the method used for calculating virus content this gives a calculated virus content of "100%", but the lower 95% confidence Interval is 29.97%. Due to this wide confidence interval, this should be interpreted as "high" virus content. In the last 2 weeks of the trial a single bulk each week was negative (19 of 20 and 39 of 40 bulks) calculated as 45% and 52.2% respectively. Given this drop in virus content is likely due to sampling variability, the results with all bulks positive are presented as 55% virus content, to avoid results being mis-interpreted.

4.3.2 Virus content in plots

Figure 4.3. Treated plot on left and untreated control plot on right showing yellowing associated with symptoms of viral infection, Photograph taken 29/06/22 (Treatment week 8).

Carrot plants were monitored for signs of infection by assessing foliar symptom development, such as yellowing and chlorotic mottling (figure 4.3). Towards the end of the trial the proportion of plants displaying symptoms in each plot was recorded on a 0-10 scale, and these were then averaged across the four plots of each treatment (figure 4.4). The untreated plot showed significantly higher levels of virus symptoms than any of the treated plots with a average symptom score of 8.0 as compared to scores between 1.0 and 2.0 for all treatments. Only one treatment displayed symptoms to a level which was significantly higher than the treated control which was the trial-long regime starting with Gazelle (T1) and then alternating fortnightly treatments of Movento (T3/T7) and Teppeki (T5/T9). Other programmes with season-long treatments including these same products but with either Movento or Teppeki as the initial treatment appeared to give better performance at limiting virus symptoms.

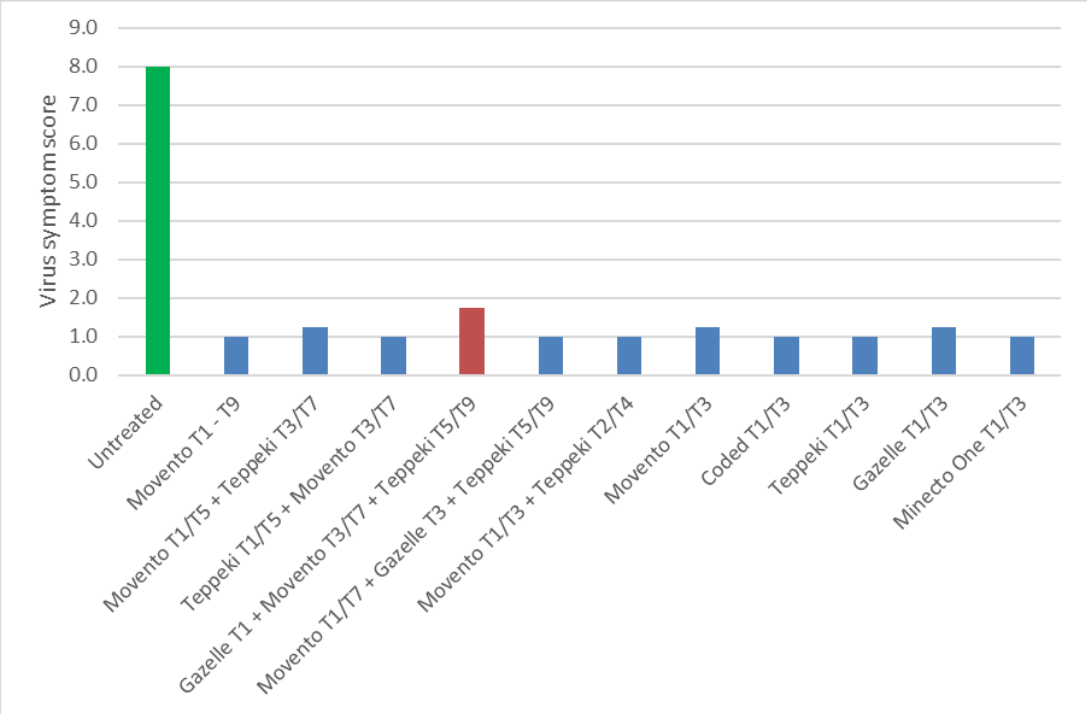

Figure 4.4. Proportion of plants displaying virus symptoms recorded in plots on a 1-10 scale. Assessed on 29/06/22. Untreated control shown in green, treated plots with a significant difference to the “treated” control (Movento T1-T9) shown in red.

The prevalence of virus in the plots was calculated from the number of bulk positive samples for each treatment regime, from sampling at the mid-point and end-point of the trial (figure 4.5). Due to the method used for calculating virus content where all bulks tested positive for virus results in a calculated virus content of “100%”, but the lower 95% confidence Interval is 29.97%. For this reason, as above, the virus content presented for “Untreated week 5” and “Gazelle T1/T3”, where all bulks tested positive, have been presented as “55%” to avoid skewing data presentation. For treatments with small changes between the week 5 test and the week 10 test, and where there is less virus in the later sampling, these differences are likely due to sampling variance. In all treatments there was a reduction in virus from the untreated control at the trial mid-point (week 5 test). Treatments with the greatest difference between initial virus suppression and control through the whole trial both had Gazelle in T1 treatment.

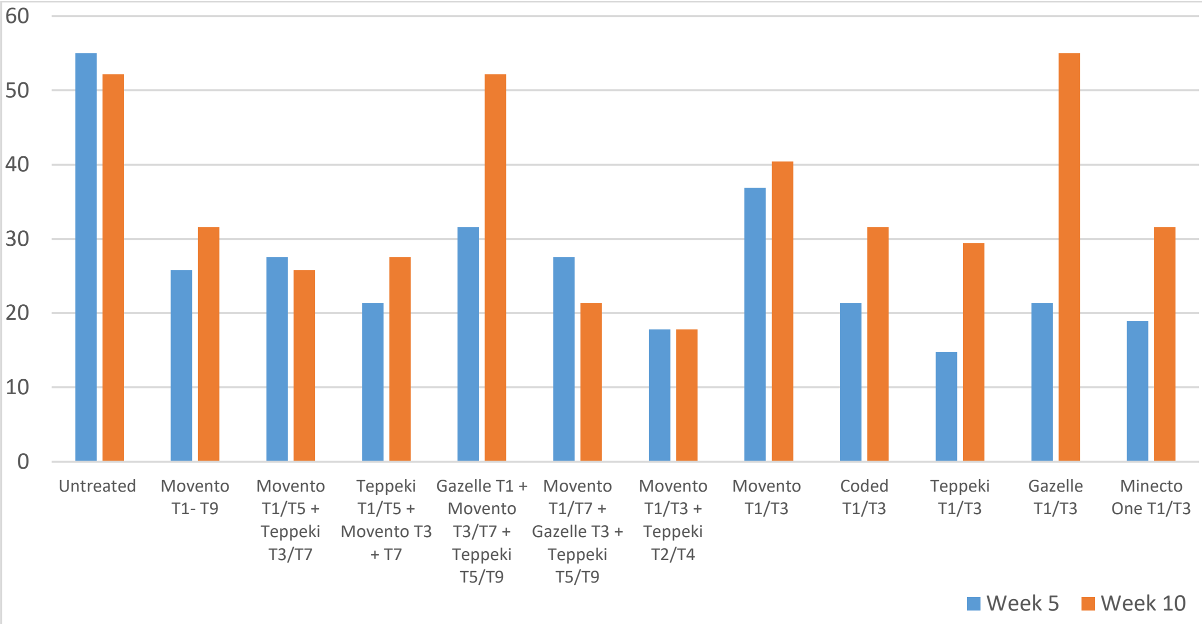

Figure 4.5. CtRLV calculated virus content per treatment. Maximum content calculated as 55% virus.

4.3.3 Yield at Harvest

Similarly, all treatments had the effect of mitigating against yield loss (Figure 4.6). Although there were little differences between treatments, the yield from some programmes were significantly lower than the treated control (intensive Movento treatment). However there does not appear to be a correlation between the virus content at mid- and end- point and the impact on yield within the trial.

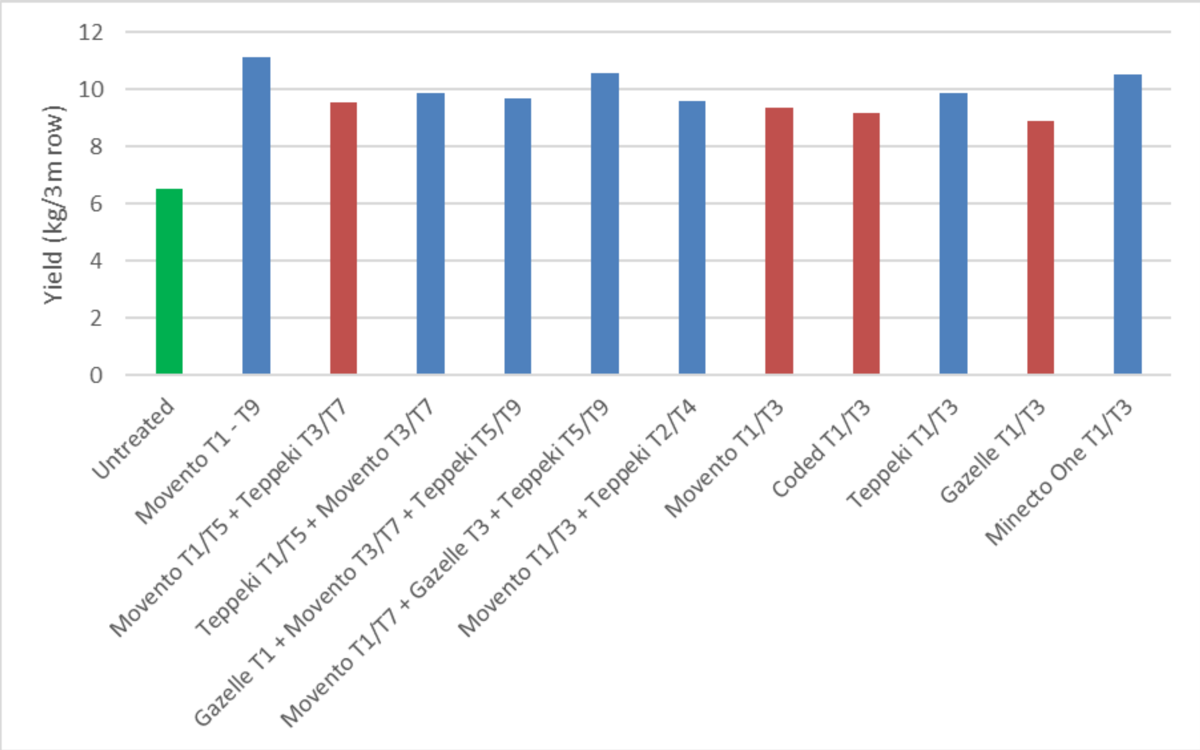

Figure 4.6. Yield of carrot roots, presented as Kg per 3 m row. Green denotes "untreated control", red where the reduction in yield was significantly reduced from treated control (Movento T1-T9).

Discussion

The 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 captures 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.

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 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 has been unknown is what contribution these aphids make in terms of virus transmission to new crops. At Wellesbourne, aphids were found regularly on overwintered carrots. These aphids 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 'confirms' that willow-carrot aphid is the key species transmitting virus, which seems likely, 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 impact of this effect over a season, by comparison to early season transmission by willow-carrot aphid is not known.

The final year of the trial focused on control measures for the virus. Given the significantly higher levels of virus transmission recorded at the Warwick site in both years of the transmission work, the trial was situated at Warwick Crop Centre, Wellesbourne. From the first week of the trial there was significant pressure from vectors, resulting in the untreated controls being heavily infected within the first three weeks of treatment programmes. All treatment programmes limited the transmission of virus into plots by comparison to the untreated control, and consequently limited the impact of virus infection on yield. There was no correlation between the treatment programmes with the best virus control and those with the least reduction in yield. These results suggest that focussing virus vector control in the early part of the growing season when virus-vector pressure is at its greatest will ameliorate the levels of virus impacting on crops, if not the overall levels of virus across the season.

The sources of viruliferous aphids in the early part of the season is still unclear. Previous work in California has suggested that incidence of carrot motley dwarf disease, the disease-virus complex associated with transmission of CtRLV, was associated with proximity to overwintering carrot crops (Watson & Falk, 1994). Monitoring of overwintering carrots within this project has also indicated that this may be a source of aphids in the early part of the growing season. However related work, funded by Defra under the Euphresco project "Baseline virus reservoirs" and using samples gathered through the BBSRC funded CALIBER project, has also revealed the presence of CtRLV and the closely related viruses wild carrot red leaf virus and Torrilis crimson leaf virus, in apiaceous weeds such as cow parsley (*Anthriscus sylvestris*). Over the course of 2023 this work will look at the distribution and hosts associated with different genotypes of these viruses at the sequence level to try to elucidate this key aspect of carrot virus epidemiology.

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*.
- 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.
- The application of chemical vector control will mitigate against the transmission of virus and its consequential impact on yield. Early season control should be the focus of control programmes to limit impact of virus infection.

Knowledge and Technology Transfer

The following activities have been used to promote the project.

- 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
- Presentation at Herb Growers Technical meeting 3 March 2023, Warwick Crop Centre
- Abstracts for presentations submitted to International Congress on Plant Pathology (Lyon, August 2023) and ISHS-International Symposium on Carrots and Apiaceae (York, October 2023)

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