Project title:	**Investigating the timing of transmission of carrot viruses to improve management strategies**
Project number:	FV460
Project leader:	Dr Adrian Fox, Fera Science Ltd
Report:	Year 1 Annual report, December 2019
Previous report:	n/a
Key staff:	Prof. Rosemary Collier, University of Warwick
Location of project:	York and Warwick
Industry Representative:	Ian Holmes, Strawsons Howard Hinds, Root Crop Ltd
Date project commenced:	January, 2019

© Agriculture and Horticulture Development Board 2020. All rights reserved

DISCLAIMER

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

© Agriculture and Horticulture Development Board [2020]. No part of this publication may be reproduced in any material form (including by photocopy or storage in any medium by electronic mean) or any copy or adaptation stored, published or distributed (by physical, electronic or other means) without prior permission in writing of the Agriculture and Horticulture Development Board, other than by reproduction in an unmodified form for the sole purpose of use as an information resource when the Agriculture and Horticulture Development Board or AHDB Horticulture is clearly acknowledged as the source, or in accordance with the provisions of the Copyright, Designs and Patents Act 1988. All rights reserved.

All other trademarks, logos and brand names contained in this publication are the trademarks of their respective holders. No rights are granted without the prior written permission of the relevant owners.

AUTHENTICATION

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

Dr Adrian Fox

Senior Plant Virologist

Fera Science Ltd

Signature .... Date ......13/1/2020............................

Prof. Rosemary Collier

Warwick Crop Centre

University of Warwick

Signature .... Date ....13/1/2020..............................

Report authorised by:

Sue Cowgill

Senior Scientist (Pests)

AHDB

Signature .... Date ....13/1/2020..............................

© Agriculture and Horticulture Development Board 2020. All rights reserved

CONTENTS

© Agriculture and Horticulture Development Board 2020. All rights reserved

GROWER SUMMARY

Headline

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

Background

Within carrot crops the key viruses of concern are carrot necrotic dieback virus; Carrot yellow leaf virus and the viruses of the carrot motley dwarf complex, the principal virus of which is Carrot red leaf virus. 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 thought to be present in high incidence for which minimal epidemiological information is available.

© Agriculture and Horticulture Development Board 2020. All rights reserved

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

Summary

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.

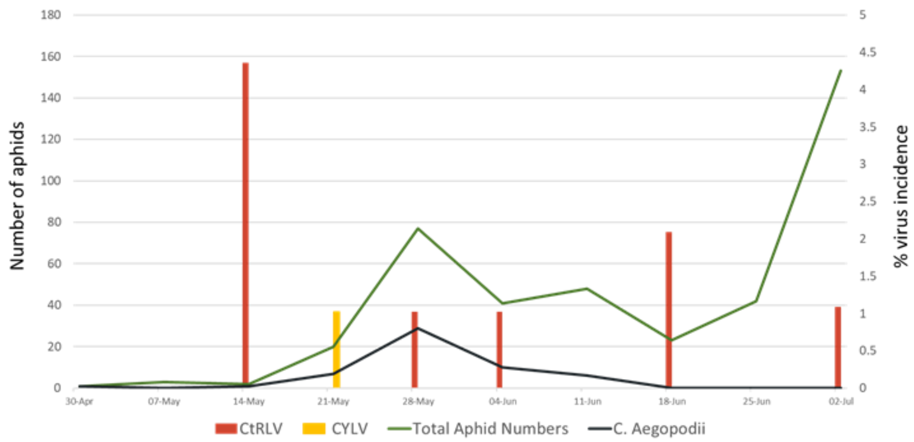


Figure1. Showing the limited virus transmission recorded at Stamford Bridge, Yorkshire. Virus content in plots is shown in the bars (Red for CtRLV, yellow for CYLV), and aphid flight data in the lines on the graph (Green for total aphid flights, Black for willow-carrot aphid).

© Agriculture and Horticulture Development Board 2020. All rights reserved

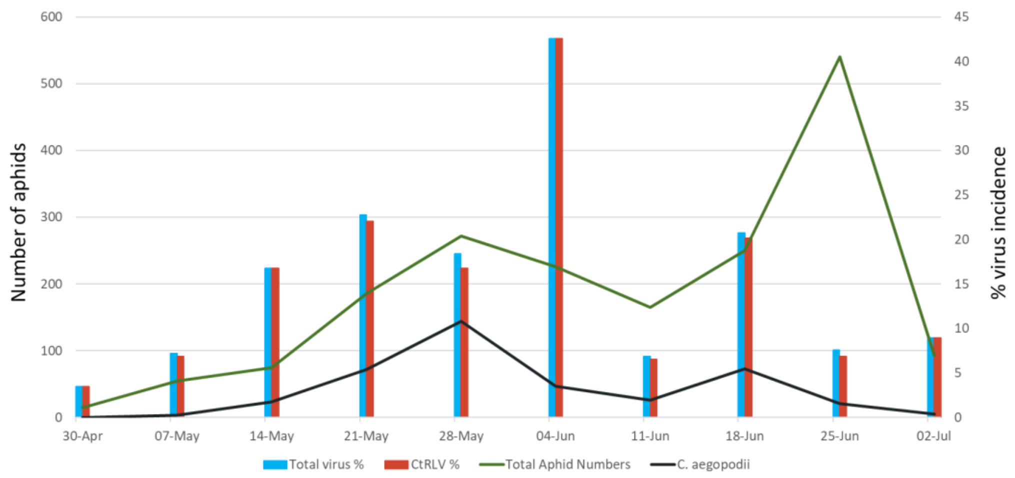


Figure 2. Virus transmission recorded in trial plots at Warwick University. Virus content is shown in the bars (Blue for total virus content, Red for CtRLV), and aphid flights in the lines on the chart (Green for total aphids, Black for willow-carrot aphid)

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*. 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.

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

Financial Benefits

As this report is based on first year data, financial benefits for growers cannot be assessed at this point.

Action Points

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

© Agriculture and Horticulture Development Board 2020. All rights reserved

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 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

© Agriculture and Horticulture Development Board 2020. All rights reserved

circulation through the aphid body. This tight relationship means that these viruses tend to be transmitted by a more limited range of vector species and transmission can take longer (at least hours) to occur. Through laboratory studies, multiple vector species may be implicated in the transmission of viruses (Naseem et al. 2016; Rozado-Aguirre et al. 2016). These studies may indicate the relative efficiency of different species, for instance previous work in potato (Lacomme et al. 2017; Fox et al. 2017a), however, this potential to transmit a virus may not directly correlate with the field epidemiology of a virus with more numerous but less efficient vectors (Lacomme et al. 2017). By examining when each vector species is migrating into a crop and correlating these data with the timing of transmission of key viruses researchers can identify both the species most closely associated with the transmission of viruses and give supporting data on the optimum time for control measures to be applied.

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

The precise timing of colonisation of crops by aphids varies from place to place and year to year and this is greatly influenced by weather conditions, particularly temperature. Potentially, as Figure 1 illustrates, there could be pressure from virus vectors (*M. persicae, C. aegopodii*) for almost 3 months. This is a long period over which to provide effective control. Thus it is important to make best use of all the information that is available on aphid phenology – both monitoring and forecasting information. At present, a basic day-degree forecast of first flight for *C. aegopodii* developed at University of Warwick is used in the AHDB Pest Bulletin. Rothamsted Research issues a forecast of the first flight of *Myzus persicae* in early March each year which is reported in AHDB Aphid News and 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, AHDB 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.

© Agriculture and Horticulture Development Board 2020. All rights reserved

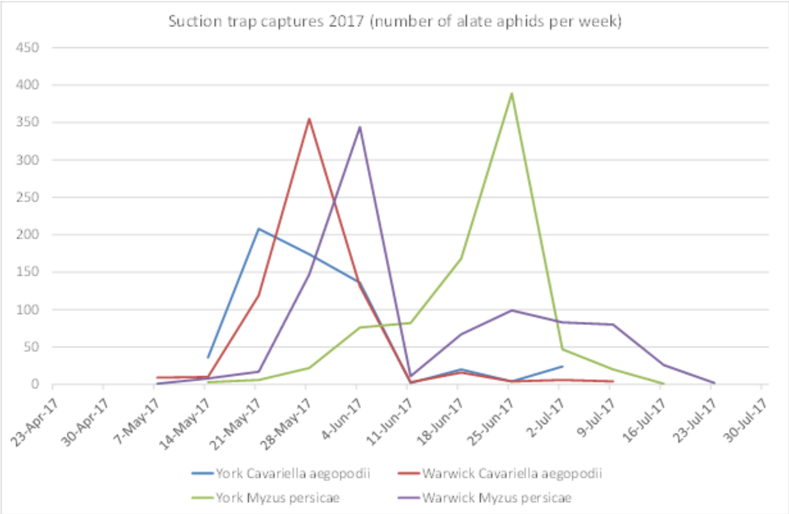


Figure 1. Numbers of alate aphids captured per week in suction traps at Fera (York) and Warwick Crop Centre (Wellesbourne, Warwick) in 2017 (data provided by the Rothamsted Insect Survey).

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

© Agriculture and Horticulture Development Board 2020. All rights reserved

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

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 will be placed close to the exposed sections. Two (2) sites used for netting trials were situated in the 'North', within a working distance of Fera and one in the 'Midlands' at Warwick Crop Centre (Wellesbourne, Warwick) (Years 1-2). Trials were set up in the week beginning 23 April 2019 and ran through to Week beginning 1 July 2019.

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

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

© Agriculture and Horticulture Development Board 2020. All rights reserved

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

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

© Agriculture and Horticulture Development Board 2020. All rights reserved

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.

Results

Captures of aphids in yellow water traps in the trial plots

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

© Agriculture and Horticulture Development Board 2020. All rights reserved

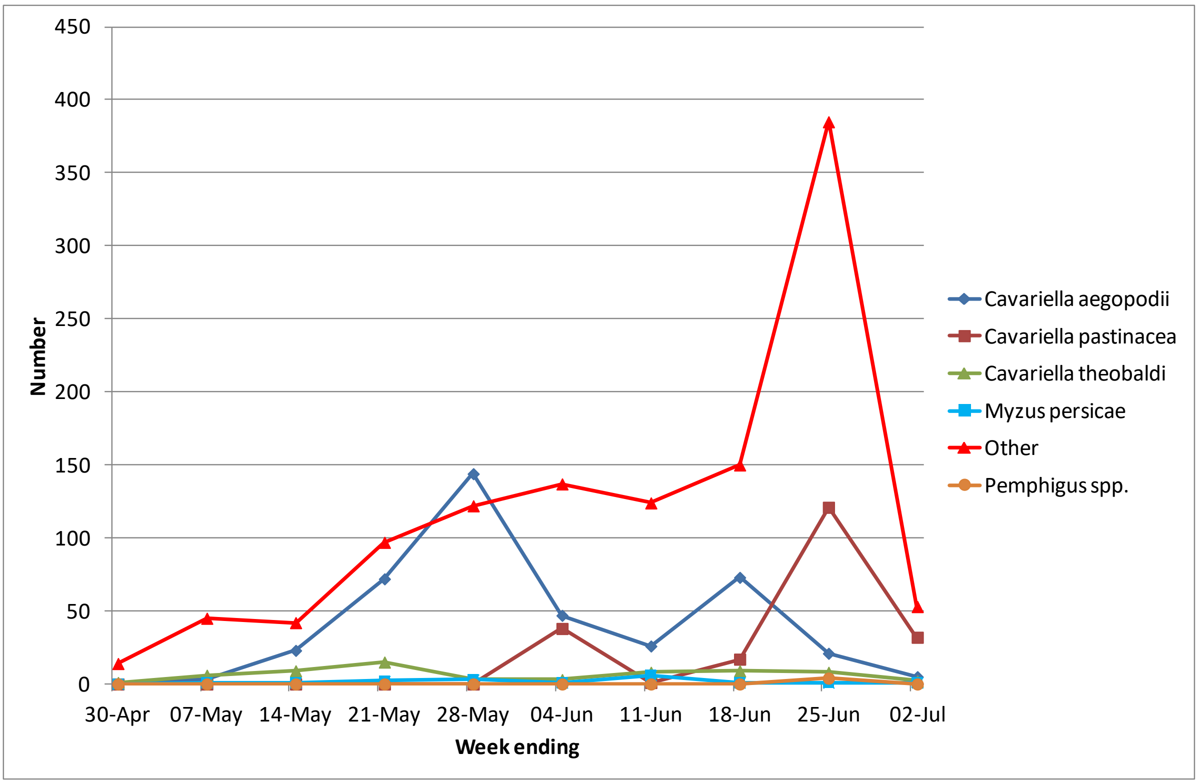


Figure 1.4 Total numbers of aphids captured per week in 4 water traps at Wellesbourne, Warwick in 2019. *C. pastinacea* and *C. theobaldii* are parsnip aphids.

Timing of transmission and correlation with vector aphids

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

© Agriculture and Horticulture Development Board 2020. All rights reserved

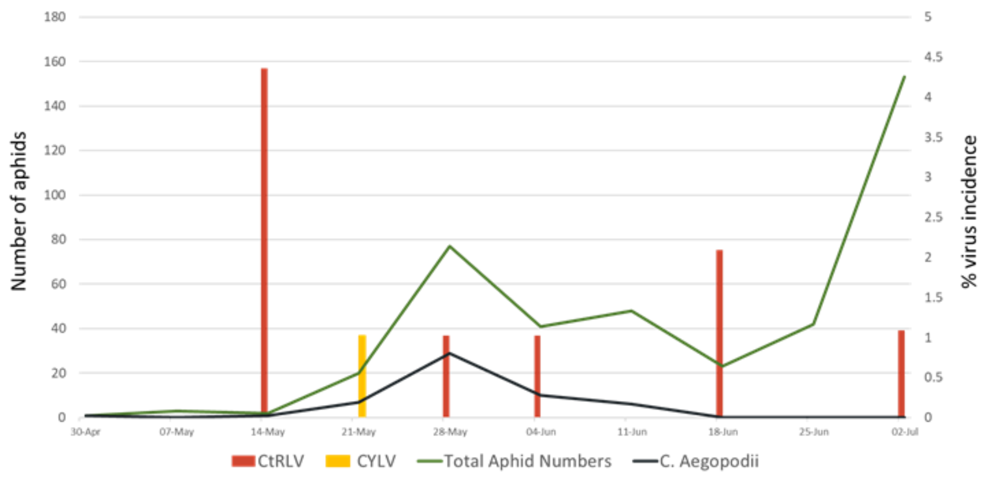


Figure 1.5. Weekly virus incidence and aphid numbers (Yellow water trap data) at Stamford Bridge, North Yorkshire. Virus data presented as % of individual viruses due to low incidence of virus transmission.

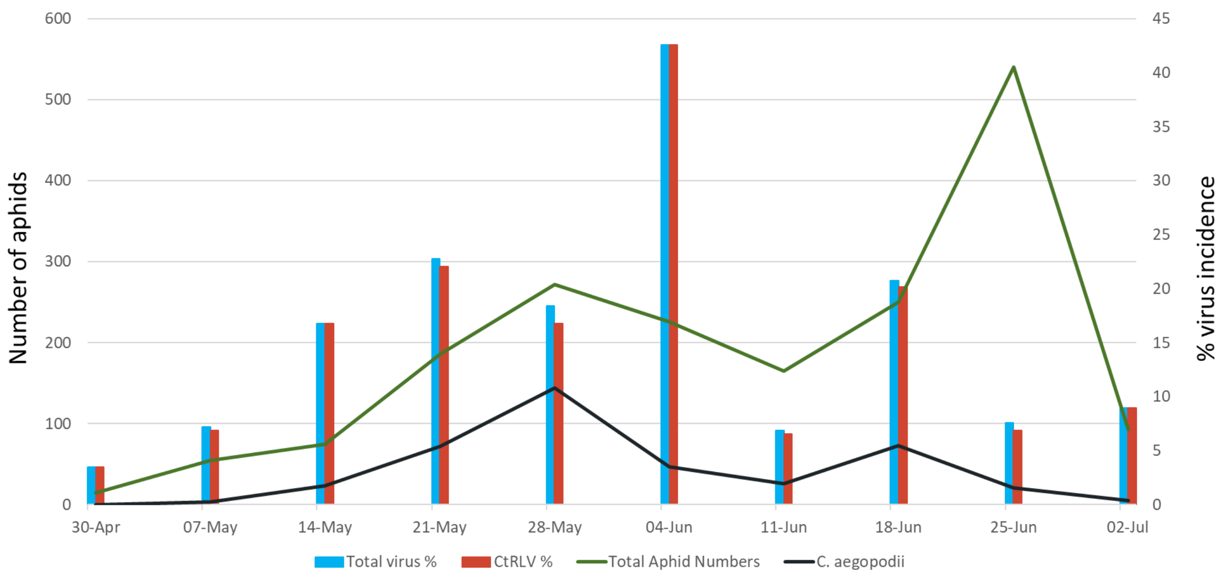


Figure 1.6. Weekly virus incidence and aphid numbers (yellow water trap data) at University of Warwick, Warwickshire. Virus data presented as % total virus and % carrot red leaf virus. Results are presented collated across all replicated plots.

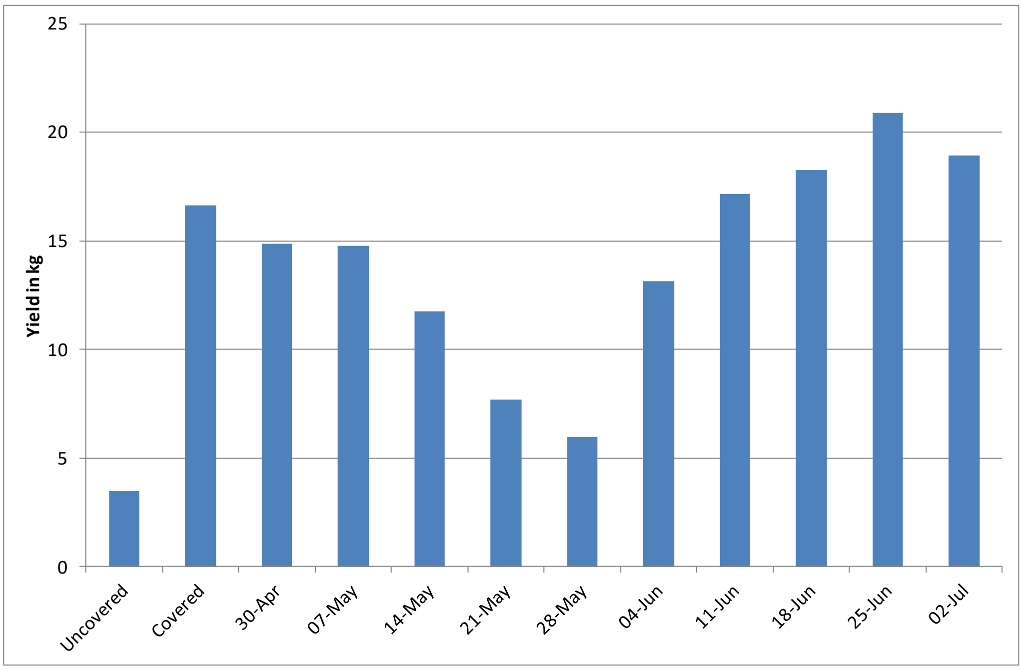
Transmission in the Warwick trial (Figure 5) was at a much higher rate than at Stamford Bridge throughout the entire season. Week to week virus incidence rose steadily through the first four weeks of the trial and after a slight drop in incidence in week 5 (28 May) recorded a peak transmission rate of just under 43% (4 June). The majority of virus transmission detected was CtRLV, with only occasional sporadic transmission of CYLV throughout the season. Plot to plot variation can be seen in the tables presented in Appendix 1.

© Agriculture and Horticulture Development Board 2020. All rights reserved

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

Yield data

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



© Agriculture and Horticulture Development Board 2020. All rights reserved

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

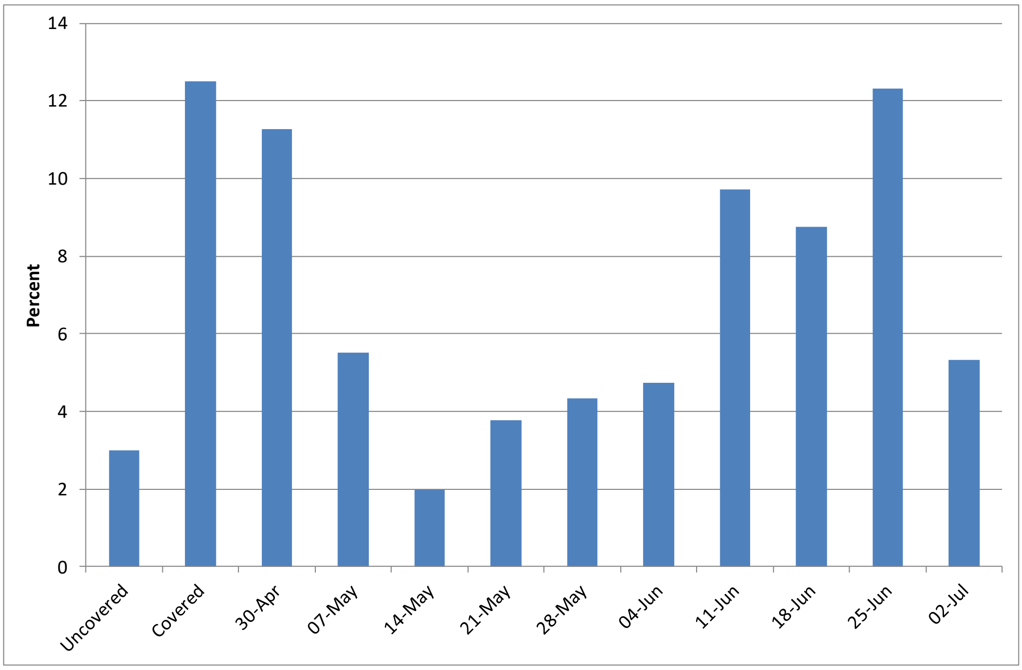


Figure 1.8 Mean percentage carrot roots undamaged by carrot fly (less than 5% of root surface damaged) (sample size 100 roots) rows) on 19-20 November 2019.

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. 20 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 from samples in accordance with previously described methods from FV432 a and b and Adams et al. (2014)

Results

20 samples of weeds were collected from both fields at Warwick and Stamford Bridge. RNA has been extracted from these samples and initial testing has been carried out to check

© Agriculture and Horticulture Development Board 2020. All rights reserved

extraction quality. Final testing of virus presence and sequence confirmation is underway and should be complete early in 2020.

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

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

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

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

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

© Agriculture and Horticulture Development Board 2020. All rights reserved

22 May	0	2	7

Aphids were also monitored on the new carrots sown in late March 2019 (Table 2). Winged aphids had arrived in the plots by 9th May and numbers of winged aphids peaked in mid to late May. Numbers of wingless aphids peaked in early June and then declined over time, there being no aphids on the plants by early August. Numbers then started to increase again in early September. Numbers of parasitized aphids peaked in mid-June and ladybirds were also present in the plots from late May – mid June.

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

Date	**Numbers of aphids and ladybird larvae**			
2019	**Winged**	**Wingless**	**Parasitised aphids**	**Ladybird larvae**
25 April	LMC 0, LMW 0	LMC 0, LMW 0	LMC 0, LMW 0	
1 May	LMC 0, LMW 0	LMC 0, LMW 0	LMC 0, LMW 0	
9 May	LMC 0, LMW 2	LMC 3, LMW 8	LMC 0, LMW 0	
14 May	LMC 4, LMW 11	LMC 1, LMW 4	LMC0, LMW 0	
22 May	LMC 22, LMW 23	LMC 8, LMW 14	LMC2, LMW 1	
30 May	LMC 25, LMW 16	LMC 27, LMW 47	LMC 2, LMW 5	LMC 1, LMW 0
6 June	LMC 21, LMW 4	LMC 391, LMW 344	LMC 2, LMW 6	
13 June	LMC 3, LMW 10	LMC 133, LMW 266	LMC 2, LMW 7	LMC 3, LMW 6
20 June	LMC 2, LMW 5	LMC 162, LMW 221	LMC 2, LMW 10	
27 June	LMC 1, LMW 2	LMC 95, LMW 143	LMC 1, LMW 9	

© Agriculture and Horticulture Development Board 2020. All rights reserved

4 July	LMC 1, LMW 2	LMC 17, LMW 18	LMC 1, LMW 1	
11 July	LMC 1, LMW 0	LMC 2, LMW 22	LMC 1; LMW 0	
17 July	LMC 0, LMW 0	LMC 5, LMW 12	LMC 0, LMW 1	
24 July	LMC 0, LMW 0	LMC 4, LMW 1	LMC 0, LMW 0	
6 August	LMC 0, LMW 0	LMC 0, LMW 0	LMC 0, LMW 0	
20 August	LMC 0, LMW 0	LMC 0, LMW 0	LMC 0, LMW 0	
4 September	LMC 1, LMW 1	LMC 21, LMW 10	LMC 0, LMW 0	
19 September	LMC 0, LMW 0	LMC 42, LMW 6	LMC 0, LMW 0	
3 October	LMC 1	LMC 38	LMC 0	

Summary data from the Rothamsted Suction traps for willow-carrot aphid 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.

© Agriculture and Horticulture Development Board 2020. All rights reserved

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

© Agriculture and Horticulture Development Board 2020. All rights reserved

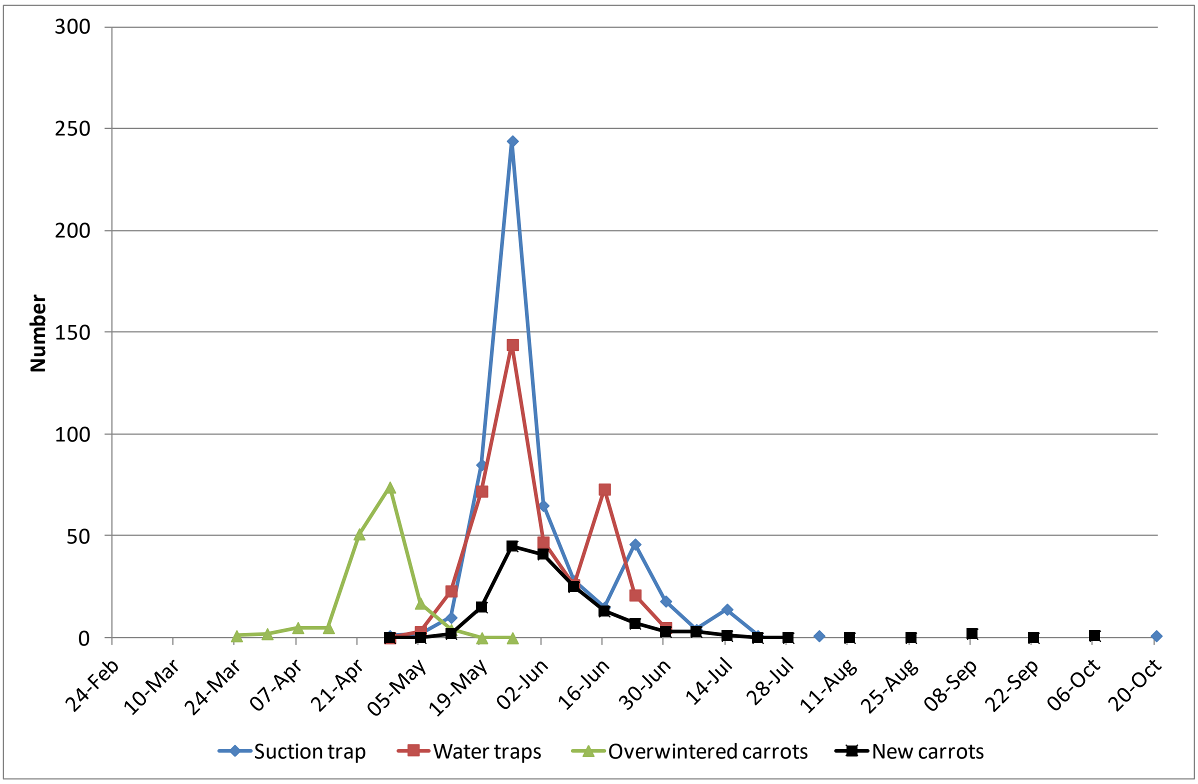


Figure 3.1 Numbers of winged willow-carrot aphid (C. *aegopodii)* captured in the suction trap, in the 4 yellow water traps in the field trial, on the plot of overwintered carrots and on plots of new carrots at Wellesbourne, Warwick in 2019.

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.

3.2 Day-degree forecasts 2019

The existing day-degree model using accumulated day-degrees from 1 February predicted the start of willow-carrot aphid flight activity at Wellesbourne to be on 30 April (Figure 3.2) (when 360 day-degrees above a base of 4.4°C had been accumulated). The first aphid was captured in the Wellesbourne suction trap by 28 April and in water traps by 7 May (samples taken weekly). The Pest Bulletin day-degree prediction for 'Yorkshire' was 14 May (weather station at Market Weighton). The first (very early) aphid was captured in the York suction trap by 21 April, but no other aphids were captured until the week ending 10th May. No aphids were captured in water traps in the Yorkshire trial until the week ending 21 May.

© Agriculture and Horticulture Development Board 2020. All rights reserved

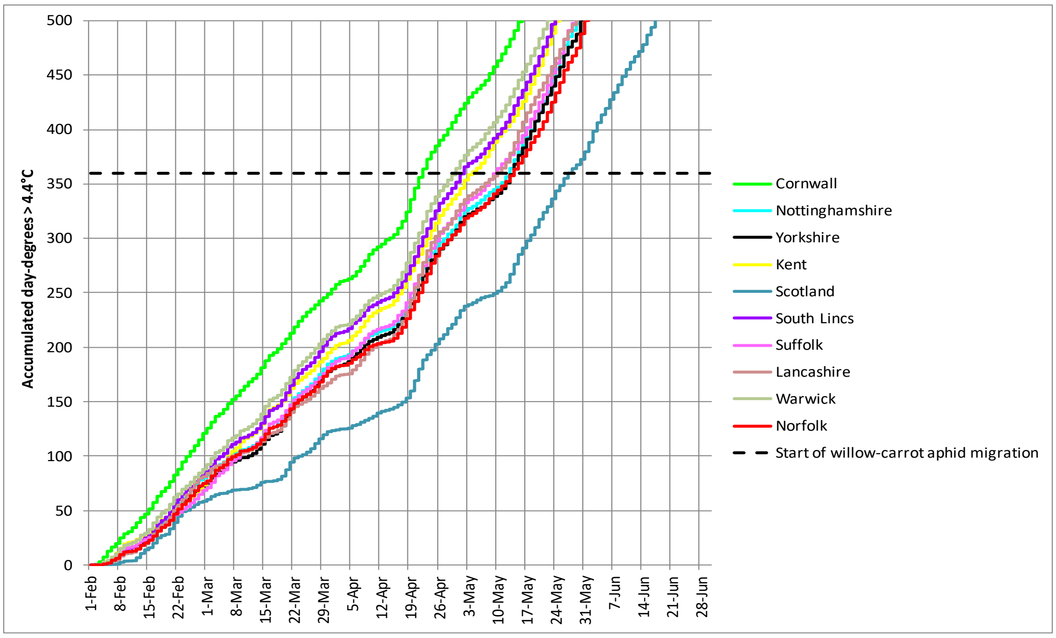


Figure 3.2. Day-degree forecasts for 2019 for the start of the willow-carrot aphid 'migration' to susceptible crops (from the AHDB Pest Bulletin). The migration is forecasted to begin when 360 day-degrees above 4.4ºC have been accumulated from 1 February.

3.3 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 3.4-3.6 and in this case the data for a region have been plotted on the same graph as the data from the nearest suction trap - as a scatter plot. Data for the Midlands and East Anglia appear to 'fit' with suction trap catches but for Grampian the water trap catches seem later than the captures in the suction trap at Dundee (the nearest functioning suction trap).

© Agriculture and Horticulture Development Board 2020. All rights reserved

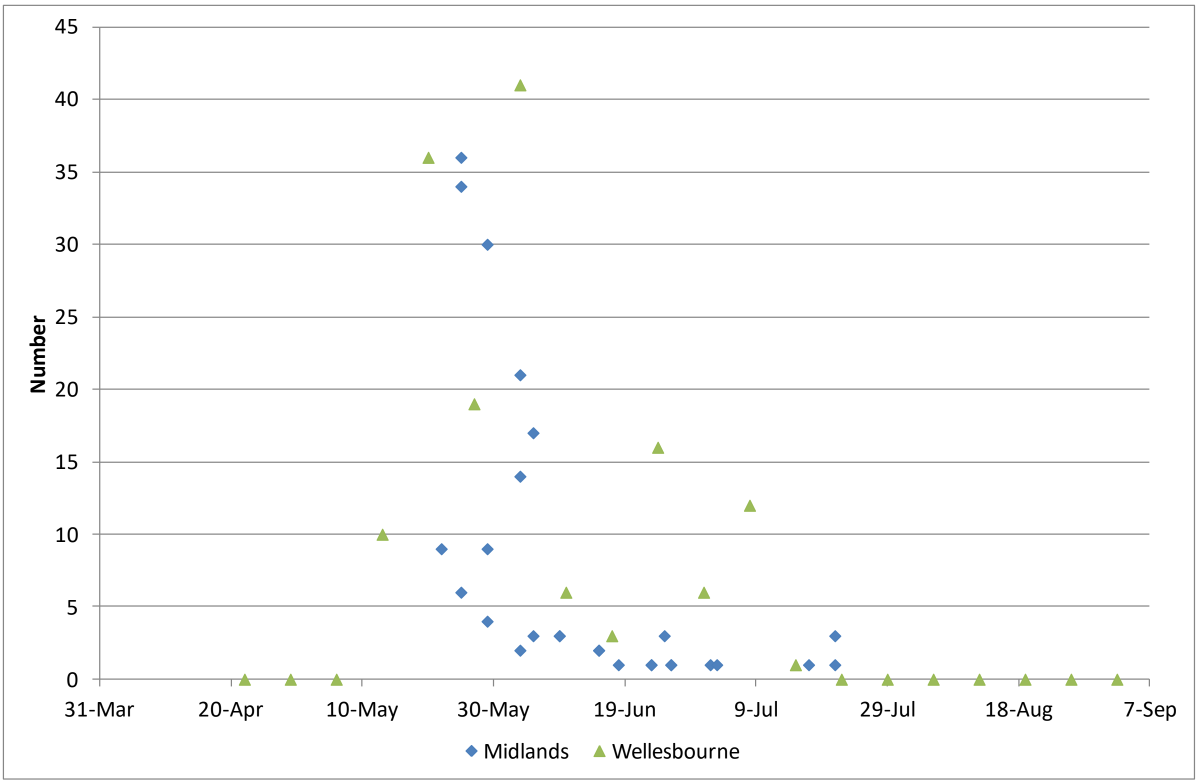
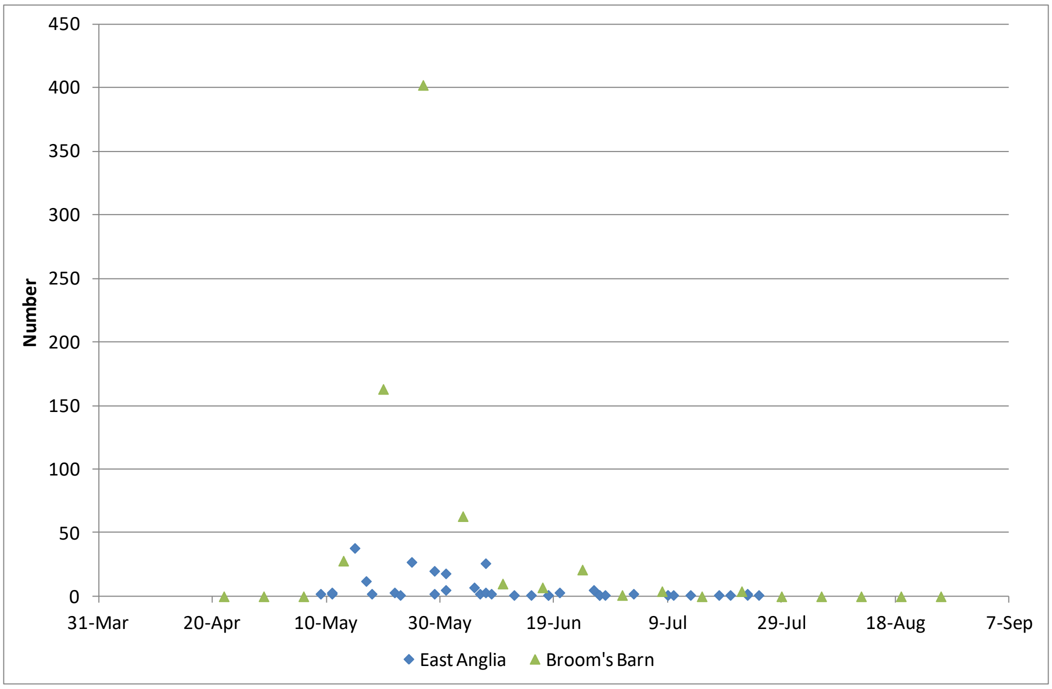


Figure 3.3 Scatter plot comparing data from Fera Science Ltd water trap samples in the Midlands region in 2018 with suction trap data from Wellesbourne.



© Agriculture and Horticulture Development Board 2020. All rights reserved

Figure 3.4. Scatter plot comparing data from Fera Science Ltd water trap samples in the East Anglia region in 2018 with suction trap data from Broom's Barn.

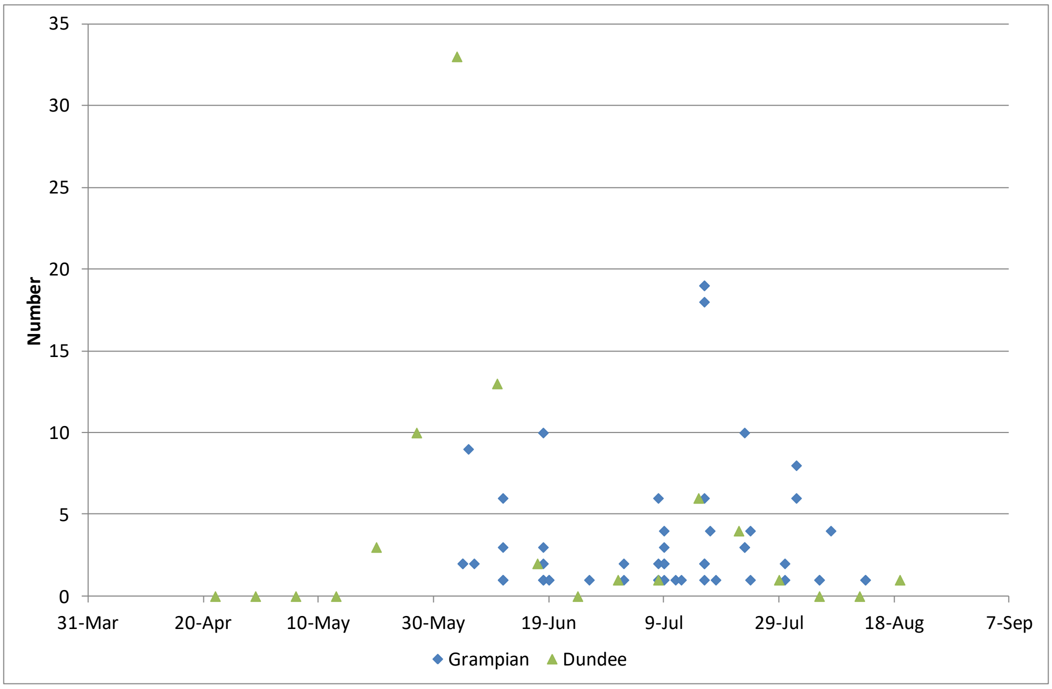


Figure 3.5. Scatter plot comparing data from Fera Science Ltd water trap samples in the Grampian region in 2018 with suction trap data from Dundee.

3.4 Relationships between suction trap data and weather data

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

© Agriculture and Horticulture Development Board 2020. All rights reserved

but these were not as robust as the day-degree forecast for willow-carrot aphid (this may be partly because there is less data).

Discussion

These field transmission data presented in this report represent the first year of a three-year project, and as such caution should be applied to interpreting too deeply from sparse datasets. That said, there is 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 a phenomenon noted in numerous virus surveys. However, within this previous carrot virus work even local differences in virus incidence were recorded, suggesting that within a region virus incidence can 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 was 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), 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.

At both sites CtRLV was the detected most commonly, 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, excep tfor 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

© Agriculture and Horticulture Development Board 2020. All rights reserved

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.

In the coming year of the project these weekly aphid flights and week-to-week virus incidences will be analysed to look for a correlation between potential vector species and timing of transmission of the viruses.

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

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

The aim of the work on monitoring and forecasting is to improve decision support for growers. The day-degree forecast for willow-carrot aphid appears to be relatively robust, whereas it may be more difficult to forecast the activity of *M. persicae* and the parsnip aphids. If this project can 'confirm' that willow-carrot aphid then this will make the provision of warnings simpler. It is also useful to know what sort of information is needed. So is it important to control the very first aphids in the crop for example?

© Agriculture and Horticulture Development Board 2020. All rights reserved

Conclusions

- Greater virus transmission was recorded in the trials at Warwick than at Stamford Bridge
- The trials at Stamford Bridge did not show a good relationship between aphid flights and virus
- The trials at Warwick had greater incidence of virus transmission throughout the season.
- Most virus detected was carrot red leaf virus at both sites, with CYLV being occasionally detected throughout the season.
- Transmission appears to track movements of *Cavariella aegopodii,* but this will be further refined in the coming seasons.
- 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.

Knowledge and Technology Transfer

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

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

© Agriculture and Horticulture Development Board 2020. All rights reserved

References

Adams, I., Skelton, A., Macarthur, R., Hodges, T., Hinds, H., Flint, L., et al. (2014). Carrot yellow leaf virus is associated with carrot internal necrosis. *PLoS One, 9*(11), e109125.

Borer, E. T., Seabloom, E. W., Mitchell, C. E., & Power, A. G. (2010). Local context drives infection of grasses by vector-borne generalist viruses. *Ecology Letters, 13*(7), 810-818.

Elnagar, S., & Murant, A. (1978). Relations of carrot red leaf and carrot mottle viruses with their aphid vector, Cavariella aegopodii. *Annals of Applied Biology, 89*(2), 237-244.

Fox, A., Collins, L., Macarthur, R., Blackburn, L., & Northing, P. (2017a). New aphid vectors and efficiency of transmission of Potato virus A and strains of Potato virus Y in the UK. *Plant Pathology, 66*(2), 325-335, doi:10.1111/ppa.12561.

Fox, A., Rozado, Z., Adams, I., Skelton, A., Dickinson, M., & Boonham, N. (2017b). Investigating the viral causes of internal necrosis in carrot. *Acta Horticulturae 1153: International Symposium on Carrot and Other Apiaceae, 1153*, 245-250, doi:10.17660/ActaHortic.2017.1153.36.

Lacomme, C., Pickup, J., Fox, A., Glais, L., Dupuis, B., Steinger, T., et al. (2017). Transmission and Epidemiology of Potato virus Y. In *Potato virus Y: biodiversity, pathogenicity, epidemiology and management* (pp. 141-176). Zurich, Switzerland: Springer.

Naseem, M., Ashfaq, M., Khan, A., Kiss, Z., Akhtar, K., & Mansoor, S. (2016). Transmission of viruses associated with carrot motley dwarf by *Myzus persicae*. *Journal of Plant Pathology, 98*(3), 581-585.

Rozado-Aguirre, Z., Adams, I., Collins, L., Fox, A., Dickinson, M., & Boonham, N. (2016). Detection and transmission of Carrot torrado virus, a novel putative member of the Torradovirus genus. *Journal of virological methods, 235*, 119-124, doi:10.1016/j.jviromet.2016.05.018.

© Agriculture and Horticulture Development Board 2020. All rights reserved

Appendix 1: Weekly data from Warwick field trial including individual plot results

Date	30-Apr	07-May	14-May	21-May	28-May	04-Jun	11-Jun	18-Jun	25-Jun	02-Jul
Week No.	1	2	3	4	5	6	7	8	9	10
Total Aphid Numbers	15	55	75	186	272	226	165	250	540	93
C. aegopodii	0	3	23	72	144	47	26	73	21	5
Total virus %	3.48	7.22	16.74	22.76	18.37	42.57	6.89	20.76	7.56	8.97
CtRLV %	3.48	6.89	16.74	22.07	16.74	42.57	6.55	20.13	6.89	8.97
% CtRLV by plot										
Plot A	2.09	8.25	21.4	8.25	12.94	36.9	4.36	18.94	1.02	14.76
Plot B	2.09	11.27	14.76	16.74	21.4	100	6.89	14.76	14.76	3.2
Plot C	2.09	6.89	18.94	45.07	6.89	100	3.2	36.9	9.71	5.59
Plot D	8.25	2.09	12.94	45.07	21.4	31.57	12.94	16.74	4.36	14.76
Total Aphid Numbers	15	55	75	186	272	226	165	250	540	93
C. aegopodii	0	3	23	72	144	47	26	73	21	5

© Agriculture and Horticulture Development Board 2020. All rights reserved