Project Title:	Parsnip Yellow Fleck Virus: development of a disease management strategy
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Key Words:	Parsnip yellow fleck virus (PYFV), anthriscus yellow virus (AYV), carrot willow aphid, *Cavariella aegopodii*

Practical Section For Growers

Background and Objectives

Background

Outbreaks of PYFV have become common in carrots with crop losses being suffered by growers throughout the UK. Infections are distributed randomly in fields with first symptoms appearing in late May and early June resulting in severely stunted plants and the death of many individual plants. Later in the season larger plants develop mottled foliage that are discoloured with yellow flecks. Plants infected with virus may develop secondary and/or misshapen roots and throughout the season infected plants can develop distinct rotting of their tops.

PYFV is detected in all stages of the crop and from carrots in storage. The virus has also been detected in symptomless plants and has recently been detected in cow parsley. Although outbreaks of the virus might appear sporadic, results from the Netherlands suggests that they can be frequent often resurging following years of little or no apparent virus.

PYFV is transmitted by the carrot-willow aphid *Cavariella aegopodii* but vectors can only successfully transmit PYFV to carrots after acquiring a co-virus, Anthriscus yellows waikavirus (AYV). With the widespread incidence of PYFV, it has been suggested that pesticides might have limited success in controlling the spread of vectors and virus as the products are not sufficiently fast acting to prevent the relatively short aphid feeding probes required for virus transmission. However, the effect of different pesticide groups on PYFV transmission is unknown and without a clear understanding of the viruses and their vectors, pesticide use to prevent damage could be indiscriminate leading to excessive insurance sprays.

Objectives

1. Determine the phenology, migration and behaviour of aphids which can transmit PYFV in carrots
2. Identify virus reservoirs and determine the acquisition, transmission, and molecular variability of PYFV and AYV
3. Develop a prototype strategy that will allow growers to implement sustainable management of PYFV and its vectors

Summary of Results

The network of water traps was re-established in 2001 throughout the major carrot growing regions in England. Aphid incidence was extremely low with only a single carrot willow aphid caught in all traps. It is intended that a similar network of traps be utilised during 2002; further data are necessary to derive suitable aphid migration forecasts. Surveys of PYFV incidence in weed and crop hosts indicated that virus levels varied with date of sample and geographical location. However, virus incidence tended to be similar or lower than in 2000/01. Further developments to TaqMan PCR assays were made to improve existing diagnostic tools. Previously collected aphids from carrot crops were analysed for the presence

of virus but all were found to be negative. Studies were undertaken to determine the effect of storage solutions on virus retention. Further investigations into the molecular variability of PYFV and AYV have been carried and additional sequencing of both viruses is underway. Laboratory experiments into the effect of temperature and transmission duration were made. Additional studies are underway to determine the optimal feeding and environmental conditions for virus transmission. Results and further understanding of vector and virus dynamics have been utilised to update a preliminary disease management strategy.

Action Points for Growers

- Disease dynamics is dependent upon vector migration. This is highly variable between regions and years and growers should consider using insect traps to monitor vector migration.
- Successful transmission of PYFV is dependent on vectors acquiring AYV from weeds. Virus reservoir status differs between weed species and the risk from PYFV is therefore, not only related to weed abundance.
- Sophisticated diagnostics tools to detect PYFV in plants and aphids provide growers/consultants with the means of assessing virus levels and thus, support informed management decisions.

Commercial benefits of the project

Development of a disease forecasting system

The forecasting system developed in this project will be able to give advance warning of potential vector population outbreaks. When used in conjunction with estimates of virus pressure (utilising the diagnostic tools and service described below) it can be used to forecast the potential risk of PYFV spread within crops which will allow consultants and farmers to plan the need, if justified, for appropriate pesticides. Whilst aphid colonisation of carrot crops can itself be a cause of concern, the damage and loss is considerably greater when aphids transmit viruses.

Development of a diagnostic service to growers and consultants

The development and implementation of practical immunological diagnostic tools for detection of PYFV within plants will additionally aid consultants and farmers to identify and quantify the extent of virus incidence within their fields and surrounding vegetation. At present they have to rely on virus symptom expression to occur before they realise that crops have become infected and by the time this is apparent it is often too late for control.

Identification of appropriate insecticide treatments and optimal pesticide application timing

Determining the effects of pesticide groups on vector dynamics and behaviour, and subsequent spread of virus will indicate the relative merits of sets of insecticides and thus, provide growers with better understanding of appropriate management options. In particular,

growers will have information on groups of pesticides that might exacerbate vector movement and virus spread.

Development of a virus management strategy

The development of a preliminary management strategy will provide a framework for synthesising the most up-to-date technology/data/information into a suitable format for use by consultants and growers to assure the sustainability and competitiveness of the industry, whilst enabling the use of insecticides on a more rational basis, especially in the event of withdrawal of key pesticides.

Towards reduced input costs and increased competitiveness

Improved understanding of the complex interactions between viruses and vectors will raise grower confidence in managing disease outbreaks efficiently, which will in turn lead to raised competitiveness and profitability of the industry. Similarly increased confidence in the ability to diagnose and forecast potential pest/disease problems and plan appropriate management strategies will reduce overall inputs and costs.

Cost-benefit analysis

Carrot production is currently worth £120 million annually to UK growers, excluding a £4 million export market. Crop losses resulting from up to 30% plant death early in the season have been reported by consultants that resulted in lack of size control with further 20% grading-out losses for early crops. National field losses are difficult to ascertain but the industry consensus of average losses in 1998 was estimated at around 4%, which is equivalent to losses of nearly £5M per annum.

Milestones

Milestones		Target date	Milestones met ?	
Number	Title		in full	on time
1/2	Monitor vector migration using water traps	01 10 01*	Yes	Yes
1/3	Derive statistical forecasts of aphid migration	01 02 02*	Yes	Yes
2/1	Test for virus incidence in potential host plants	01 10 01*	Yes	Yes
3/1	Develop prototype disease forecasting system	01 02 02*	Yes	Yes
3/3	Develop preliminary management strategy	01 02 02*	Yes	Yes

*Annual milestones with target dates for the first year indicated

Staff Effort

Organisation	Grade	Years
CSL	Band 6	0.2
	Band 5	0.6
	Band 2	0.8
HRI	Research Leader	0.4
	Research Assistant	1.5
ADAS	Senior Consultant Statistician	0.01
	Senior Consultant Entomologist	0.12
	Consultant Entomologist	0.08
	Senior Scientific Officer	0.28
	Scientific Officer	0.16

SCIENCE SECTION

Background

The anthriscus strain of parsnip yellow fleck sequivirus (PYFV) has become epidemic in carrots with crop losses being suffered by growers throughout the UK. Early carrot crops infected with virus can be severely stunted and many individual plants die. Infections are distributed randomly throughout fields with first symptoms appearing in late May and early June. Later in the season larger plants develop mottled foliage discoloured with yellow flecks. Plants infected with virus develop secondary and/or misshapen roots and throughout the season infected plants can develop distinct rotting of their tops.

PYFV is detected in all stages of the crop and from carrots in storage. The virus has also been detected in symptomless plants and has recently been detected in cow parsley. Although outbreaks of the virus might appear sporadic, results from the Netherlands suggests that they can be frequent often resurging following years of little or no apparent virus.

PYFV is transmitted by the carrot-willow aphid *Cavariella aegopodii* but vectors can only successfully transmit PYFV to carrots after acquiring a co-virus, Anthriscus yellows waikavirus (AYV). With the widespread incidence of PYFV, it was clear that pesticides had limited success in controlling the spread of vectors and virus because the products used were not sufficiently fast acting to prevent the relatively short aphid feeding probes which are adequate for virus transmission. However, the effect of different pesticide groups on PYFV transmission is unknown. Without a clear understanding of the viruses and their vectors, pesticide use to prevent damage could be indiscriminate and lead to excessive insurance sprays.

Scientific Milestones for Year 2

- Monitor vector migration using water traps
- Derive statistical forecasts of aphid migration
- Test for virus incidence in potential host plants
- Develop prototype disease forecasting system
- Develop preliminary management strategy

Summary of work completed in Year 2

Monitor vector migration using water traps

The network of water traps established in 2000 was expanded upon with sites utilised throughout the major carrot growing regions in England (Figure 1). Aphids caught in the traps were sent to the ADAS laboratory for identification. However, aphid incidence was extremely low with only a single carrot willow aphid caught in all traps over the 10-week trapping period. No other *Cavariella* spp were caught. With the exceptionally low aphid numbers caught in the water traps it was decided to extend aphid monitoring for a further two weeks. However, no *Cavariella* spp were caught during this extension. Similar results were

observed other trap networks with markedly fewer carrot willow aphids caught during 2001 than 2000 (Table 1). It is intended that a similar network of traps be utilised during 2002 and the data utilised with existing results to derive suitable aphid migration forecasts.

Derive statistical forecasts of aphid migration

The derivation of statistical forecasts of aphid migration is on-going. However, because of the extremely low numbers of carrot willow aphids caught in 2001, it has not been possible to update existing relationships. It is intended that a similar network of traps be utilised during 2002 and the resulting data will be used to extend the existing databases and forecasting relationships.

Test for virus incidence in potential host plants

Samples of umbelliferous crops and potential weed hosts in fields with water traps was made weekly during the aphid migration period. A total of 15 to 20 plant samples were taken per week whereby five or six leaves were taken from an individual host. Samples were collected by ADAS and sent to HRI-Wellesbourne. Virus levels in wild and cultivated host plants (as carried out during 2000/01) were analysed using ELISA. PYFV incidence was lower in carrots in 2001/02 than in 2000/01 (18% and 30%, respectively), while incidence in cow parsley was similar in both years (around 40%). The incidence of PYFV was very low in all other weed hosts except for rough chervil where over half of the samples tested were infected. Virus incidence changed as the season progressed for different hosts, although it remained relatively constant for cow parsley samples. Furthermore, virus incidence varied with geographical location with a site in Cambridgeshire having the highest disease levels.

Develop prototype disease forecasting system

Aphids caught in water traps in 2000 and 2001 have been stored at CSL. Sub-samples of the aphids were chosen randomly from both years, and the presence of AYV and PYFV detected utilising TaqMan PCR. Initial analysis focused on the detection of RNA, as an indicator of the presence of virus, however, it was not discovered in any of the samples tested. A further study was undertaken to determine the effect of storage solution on RNA extraction. Viruliferous aphids were stored in five solutions, distilled water, 70 and 100% ethanol, 50% methanol and RNA Later, for one, two or six weeks and the presence of RNA determined using TaqMan PCR. Results indicated that either 70 or 100% ethanol were the best storage solutions with RNA extracted from all vectors tested (Figure 2). RNA Later and 50% methanol were less effective, and no RNA was detected from vectors stored in distilled water.

Molecular variability of AYV and PYFV

Work has continued with the sequencing of both PYFV and AYV.

For PYFV a small section of the polymerase gene (A = 450nt) and a small section of the helicase domain (B = 179nt) had been amplified previously using PCR and degenerate primers designed to closely related viruses (Figure 3). In each case the PCR product had been cloned and sequenced. Using primers designed within the polymerase gene and the helicase gene, long range RT-PCR has been used to amplify the region between these two conserved

domains (C in Figure 2). The resulting PCR products have been cloned. Sequence is currently being generated for the clones, this should give 4000nt (approx) of sequence in total.

For AYV further primers have been designed within the region previously sequenced, and a technique known as 3 ′ RACE (Rapid Amplification of cDNA Ends) was employed to amplify the region between the poly-A tail (present in AYV but not PYFV) and the polymerase region already sequenced. The resulting PCR product was cloned and sequenced resulting in the elucidation of a further 1673nt of the AYV genome (Figure 4).

AYV:COX multiplex and PYFV simplex assay development

Primers and probes have been designed to sequence within the polymerase gene region for both AYV and PYFV. For PYFV a multiple sequence alignment of >15 isolates of the virus was designed (Table 2), while a single isolate from cow parsley was used for AYV. Each assay was subjected to a primer optimisation matrix and the optimum concentration selected in each case. A previously designed 'universal plant' assay to the cytochrome oxidase gene (COX) was also optimised and subjected to primer limitation in order to allow multiplexing with the AYV assay. Comparisons were then made with the AYV/COX assay to ensure that they performed equally well in multiplex as well as simplex formats. Thus assays can be performed for both AYV and PYFV from plant material with an internal positive control in two assays.

Acquisition and transmission of AYV and PYFV

Preliminary studies were undertaken to determine the effect of temperature and inoculation time on the transmission of AYV. Experiments were conducted at two temperatures, 10ºC and 20ºC, and four inoculation access periods (IAP), 2, 10, 30 minutes and 24 hours. Five alate viruliferous *C. aegopodii* were transferred to each of 30 *Anthriscus cerefolium* seedlings for each IAP. Aphids were sprayed off with 'Rapid' aerosol after each IAP, the seedlings were returned to a glasshouse cubicle and maintained at 18ºC. After a further three weeks, all seedlings were extracted for RNA using the CTAB extraction method and tested for virus using the AYV/COX multiplex TaqMan® assay. Results indicated that AYV transmission was dependent upon IAP and temperature (Figure 5); transmission increased with increasing temperature and IAP, although it appeared to decline when aphids were allowed an IAP of 24 hours at 20ºC. Further studies are planned to investigate additional temperatures and IAP, and using single vectors to determine transmission probabilities.

Develop preliminary management strategy

An extensive search of the scientific literature was undertaken to gather all relevant information/knowledge. The results of this wide-ranging search was integrated with results from current studies and used to formulate part of a draft management strategy. Action points for growers/consultants derived are: disease dynamics is dependent upon vector migration. This is highly variable between regions and years and growers should consider using insect traps to monitor vector migration. Successful transmission of PYFV can only occur if vectors have acquired AYV from alternative hosts. Virus reservoir status differs between host weed species and risk from PYFV is therefore, not only related to weed abundance. Sophisticated diagnostics tools to detect PYFV in plants and aphids provide

growers/consultants with the means of assessing virus levels and thus, support informed management decisions.

Technology Transfer

Close informal interaction between consortium partners and with growers in general has occurred throughout the reporting year. Samples of aphids from the networks of water traps and suspected virus infected plants have been received from several sources, and the results of studies utilising these materials have been presented to and discussed with the originators. In addition several visits have been made to growers' farms and discussions regarding the project have taken place.

Presentations

Julie North presented a poster 'Development of TaqMan® Assays for the Detection of Parsnip Yellow Fleck Virus and Anthriscus Yellows Virus' at The RES Postgraduate Forum, London, 2002

Derek Morgan presented a poster 'PYFV: Development of a Management Strategy' at The UK Onion and Carrot Conference & Exhibition, Spalding, 2001

Tables

	Year	
Site	**2000**	**2001**
Askham Bryan, Yorks	205	11
Preston, Lancs	323	9
Kirton, Lincs	470	150
Brooms Barn, Suffolk	943	44

Table 1 Number of carrot willow aphids caught at four Rothamsted suction trap sites

ISOLATE	F PRIMER	3' END	PROBE	5' END	R PRIMER	3'end	TOTAL
489 C	1	0	2	0	3	0	6
486 B1	1	0	2	0	3	0	6
489 e2*	1	0	1	0	3	0	5
519 E	1	0	4	0	3	0	8
519 G	1	0	2	0	2	0	5
486 B4*	0	0	0	0	3	0	3
489 H	0	0	0	0	2	0	2
V2005207**	2	0	3	0	2	0	7
527 F	2	0	4	0	4	0	10
wis-C*	1	0	2	0	3	0	6
V2003614**	0	0	2	0	4	0	6
CV065	2	0	5	0	3	0	10
508	3	0	5	0	3	0	11
P121	3	0	5	0	3	0	11
CV 506	1	0	9	0	4	0	14
513 C	3	1	5	0	5	0	13
513 B	3	1	5	1	5	0	13
A421	3	0	6	0	4	0	13
516 B	0	0	4	0	4	0	8
515 A	0	0	6	0	2	0	8
4266**							
4242							
518 A							

Strong +ves * Not tested

Weak +ves ** - ve COx

Table 2. Singleplex assay against PYFV isolates

Figures

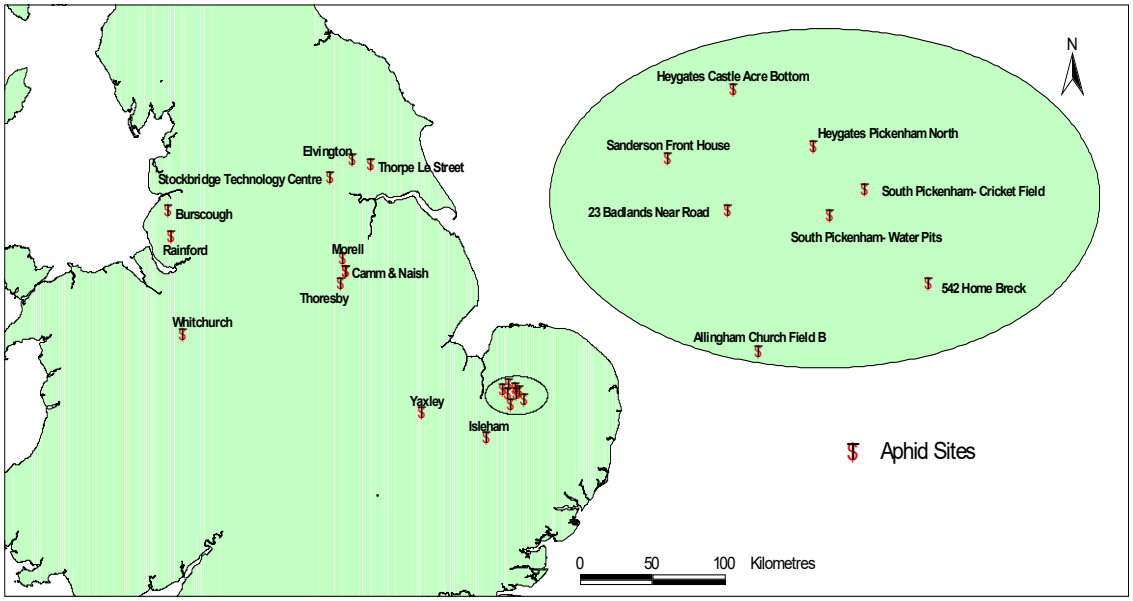

Figure 1. Map showing aphid monitoring locations

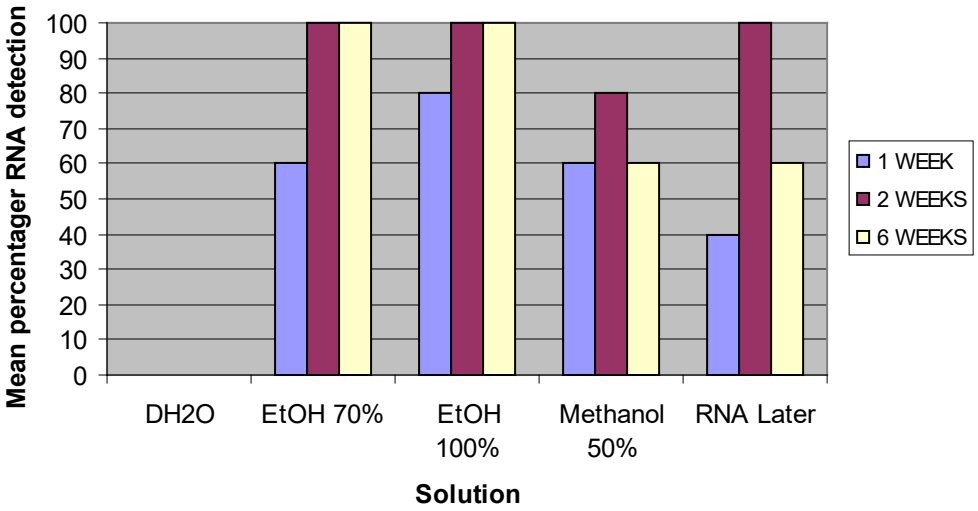

Figure 2. Effect of storage solution and duration on RNA extract from viruliferous aphids

PYFV genome approx 9000nt

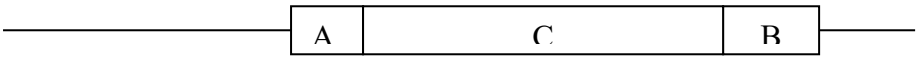

Figure 3. Diagrammatic representation of PYFV genome and the regions sequenced (boxed)

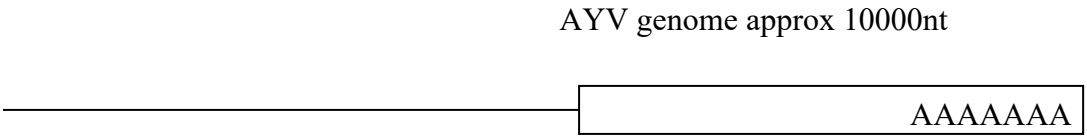

Figure 4. Diagrammatic representation of AYV genome and the region sequenced (boxed)

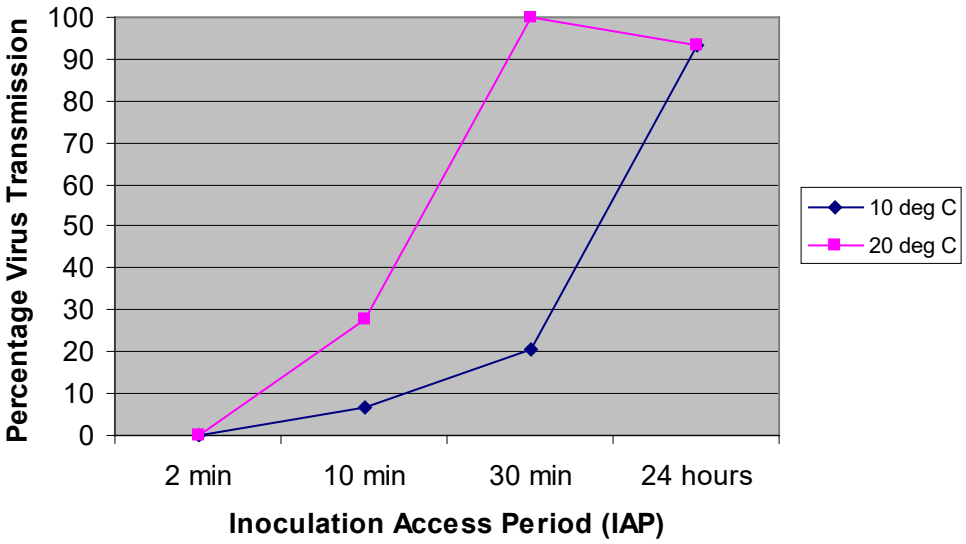

Figure 5. Effect of temperature and inoculation time on transmission of AYV