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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 which 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

Networks of water traps were established throughout the major carrot growing regions in England. Traps were sampled frequently, all aphids caught were sent to the laboratory for identification and results were returned to growers and consultants within 48 hours of receiving the samples. Results indicated that the carrot willow aphid was the most common species of colonising aphid, especially early in the growing season, and aphids tended to be more abundant at sites in the east Midland and Yorkshire than those in Lancashire and east

Anglia. In addition, the range and abundance of aphid species that are not considered to colonise carrots were extensive and often outnumbered colonising species. It is intended that a similar network of traps be utilised during 2001.

A culture of the carrot-willow aphid *Cavariella aegopodii* was established under controlled environmental conditions on carrot plants. Host suitability studies indicated that the aphids performed better when reared on parsnip than on spinach, requiring only 15 days to develop from birth to adulthood at 20ºC. Further studies are planned to investigate the role of host plant in vector dynamics.

Surveys of potential virus host plants sourrounding carrot fields were undertaken whereby the abundance of umbelliferous weeds was recorded. Results indicated that there was large variation in the abundance of possible weed hosts but some plants were present at all sites. Samples of crop and weed host plants, from fields with aphid water traps, were sent to the laboratory for virus detection using ELISA, developed in a previous HDC-funded project (Spence *et al*., 2000), and results indicated that around 20% of carrots and over 40% of weeds were infected with PYFV. In addition innovative molecular tools were developed to characterise virus isolates and results indicated that PYFV from cow parsley and carrot were related but distinct from isolates originating in celery and parsnip which in turn were related to each other. Studies are underway to characterise the viruses further and will continue during 2001.

Action Points for Growers

- Aphid migration is highly variable between regions and years. Consider using yellow water traps to monitor aphid migration
- Successful transmission of PYFV can only occur if vectors have acquired AYV from umbelliferous weeds. Crops are potentially at more risk in areas with high weed abundance.
- Sophisticated diagnostics tools to detect PYFV in plants have been developed. Consider utilising these tools to determine crop virus levels accurately.

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 which 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
1/1	Identify aphids from existing water trap networks	01 05 00	Yes	Yes
1/2	Monitor vector migration using water traps	01 10 00*	Yes	Yes
1/4	Investigate aphid alighting on plants	01 11 00*	Yes	Yes
2/1	Test for virus incidence in pootential host plants	01 10 00*	Yes	Yes

* Annual milestones with target dates for the first year indicated

Staff Effort

Organisation	Grade	Years
CSL	Level 4	0.2
	Level 3	0.6
	Level 2	0.8
	Level 1	1.2
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 (Tyler, 1998). 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 (Tyler, 1999).

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 (Spence & Wright, pers. comm.). 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 (van Dijk & Bos, 1985).

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) (Elnagar & Murant, 1976a). 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 (van Dijk & Bos, 1985). 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 1

- Identify aphids from existing existing on-going trap networks
- Monitor the phenology and migration of aphid vectors into carrot crops
- Investigate alate aphid alighting on plants and between-plant movement under controlled conbditions
- Collect potential hosts from margins of carrot crops and test for virus incidence using ELISA

Summary of work completed in Year 1

Networks of aphid water traps were established in fields throughout the major carrot growing regions of the UK. Trap catches at most sites indicated that carrot willow aphid was most abundant from mid May to the end of June but numbers declined as the season progressed. The highest number of colonising aphids were caught in the east Midlands and Yorkshire rather than in Lancashire and east Anglia. Moreover, significant numbers of non-colonising

aphids species were caight in the traps. A culture of carrot willow aphids was established and investigations into host plant suitability have begun. A botanical survey at two field sites was carried to determine the incidence of potential umbelliferous weed reservoirs. Weed diversity was low although bur chervil, wild celery and cow parsley were found in abundance. Samples from umbelliferous crops and weeds throughout the UK were tested for the presence of PYFV using antibodies developed in a previous HDC project. A significant proportion of samples from carrots, celery, parsnips and cow parsley were found to contain PYFV (18%, 17%, 30%, and 40%, respectively), although no virus was detected in the other weed hosts. The molecular characterisation of PYFV and AYV has begun; contrasts between PYFV and AYV, and closely and remotely related viruses were made. Results indicated that PYFV extracted from cow parsley and carrot were related to each and distinct from virus found in celery and parsnip which were closely related to each other.

Materials and Methods

Identify aphids from existing on-going trap networks

A network of water traps was established at commercial field sites in England. At each site a rectangular yellow water trap (400mm x 300mm) was erected on a south or southwest headland approximately 5m into the crop. Traps were sampled twice weekly for 10 weeks whereby the trap collecting fluid was strained though a muslin sheet and any insects caught were collected and returned to the laboratory for identification. The trap catches were sorted using a binocular microscope and aphids of *Cavariella* spp. isolated and identified to species. Other aphid species were collected and stored in 70% alcohol for identification at a later date.

Monitor the phenology and migration of aphid vectors into carrot crops

Field sites were selected on the basis of either a history of PYFV or of known infestations of willow-carrot aphid. The methods used to in the previous section were followed to establish and service traps.

A second network of water traps was established utilising facilities provided by the industrial partners of the project consortium. Circular yellow water traps (30cm diameter, 15cm deep) were erected in the centre of carrot crops at crop height. Traps were sampled by industrial partners as often as possible and all insects collected were sent to the laboratory. All aphids were identified to species and results were relayed back to the industrial partners within 48 hours of receiving the samples.

Investigate alate aphid alighting on plants and between-plant movement under controlled conditions

Preliminary studies of host plant suitability and *C. aegopodii* were undertaken at 20ºC between parsnip, *Pastinaca sativa*, and spinach, *Spinacia oleracea*. Alate adult aphids were clip caged onto individual leaves and allowed to reproduce. After 24 hours the adult and all offspring except one were removed and the remaining individual monitored daily until it became reproductively mature. Survivorship and time elapsed between birth and adulthood were recorded.

Collect potential hosts from margins of carrot crops and test for virus incidence using ELISA

Samples of umbelliferous crops and potential weed hosts in fields with water traps was made weekly during the aphid migration preiod. Plant samples were selected at random and all were tested for PYFV infection using ELISA, while all samples of cow parsley were also tested for the presence of AYV using PCR (see section below). In addition industrial partners of the consortium were encouraged to send samples of crops they susepcted to be infected with PYFV and their virus status was confirmed and the results relayed back to the appropriate partners.

A botanical survey of potential weed hosts was conducted at three sites. Field margins and hedgerows sourounding fields were sampled for the presence of umbelliferous weeds and the relative frequency/cover of plant species present was assessed using the DAFOR abundance scheme. At all sites field boundaries were assessed and sketch maps drawn.

ELISA for routine detection of PYFV was further modified and developed using antibodies produced in HDC project FV 228 "Carrots: diagnosis of PYFV". Small quantities of antisera prepared against the parsnip strain of PYFV (P121) and the anthriscus strain of PYFV (A421), which were produced in the early 1980's, were obtained from SCRI for comparison. Freeze-dried leaf material infected with virus strains A121 and P421 were also obtained and established in culture by mechanical inoculation to *Nicotiana* spp.. Samples from these cultures were used as reference strains for subsequent serological and molecular studies. Purification of PYFV was continued to underpin further monoclonal antibody production.

Samples of umbelliferous weeds and crops were received for PYFV detection and ELISA was used in combination with electron microscopy and sap inoculation to *Chenopodium quinoa* and Nicotiana species to determine virus presence.

DNA alignments were made from the peptide sequences of all the characterised members of the *Sequiviridae*. Any areas of sequence similarity identified were then aligned using the nucleotide sequence. Areas of nucleotide sequence homology were identified within the polymerase gene between the following viruses: parsnip yellow fleck (parsnip strain), rice tungro spherical virus, maize chlorotic dwarf virus and the Comovirus cowpea severe mosaic virus which was found to be similar to members of the Sequiviridae within the polymerase region.

Following alignment of the polymerase region, areas of sequence homology were identified and eight degenerate PCR primers were designed (four forward and four reverse). The primers designed could be used in combination to amplify between 122 and 527 nucleotides of the polymerase gene (Figure 1; Table 1).

Results and Discussion

Identify aphids from existing on-going trap networks

Several species of aphids were caguht in the water traps in carrot crops. Although aphids species known to be colonisers of carrot crops, *C. aegopodii*, *C. theobaldii* and *C. pastinacae*, were caught frquently, other species of aphids (non-colonisers) were also abundant. Examples included the bird cherry oat aphid, *Rhopalosiphum padi*, potato aphid,

Macrosiphum eurphorbiae, black bean aphid, *Aphis fabae*, cabbage aphid, *Brevicoryne brassicae*, grain aphid, *Sitobion avenae*, roas aphid, *Macrosiphum rosae*, vetch aphid, *Megoura viciae*, and the nettle aphid, *Macrosiphum evansi*.

Monitor the phenology and migration of aphid vectors into carrot crops

Nine field sites were established in the major umbellifer crop growing regions in England (Table 2). Of the *Cavariella* spp. caught in the traps, *C. aegopodii* was most abundant and occurred earliest in all regions (Figures 2-10). At all sites the total number of *C. aegopodii* caught was greater than the total of other *Cavariella* spp.. The profile of *Cavariella* spp. varied with crop; in carrot fields the dominant *Cavariella* spp. was *C. aegopodii* while in parsnip fields it was either *C. theobaldii* or *C. pastinacae*. Regional variation in *C. aegopodii* numbers was evident; aphids were more abundant in the east Midands and Yorkshire regions than in north west England and east Anglia (Figures 2-10). However, at most sites the total number of non-*Cavariella* spp. was greater than *Cavariella* spp..

Traps were established on sites provided by consortium partners (Table 3). Of the *Cavariella* spp. caught in the traps, *C. pastenacea* was most abundant (Figures 11-14) but occurred much later into the crop growing season than *C. aegopodii* (Figures 11-14). Regional variation in trap catch numbers was evident with most *Cavariella* spp. being caught in the south west Midlands region and least in Lancashire (Figures 11-14). At most sites the total number of non-*Cavariella* spp. was greater than *Cavariella* spp..

Investigate alate aphid alighting on plants and between-plant movement under controlled conditions

Samples of *C. aegopodii* were received from IVEM and a culture of aphids was established and maintained on carrot under controlled environment conditions. Preliminary results indicated that aphids performed better when reared on parsnip than on spinach; all of the aphids reared on parsnip developed through to adulthood, while around 25% of those kept on spinach died. The development time on parsnip from birth to adulthood took 15.25±0.95 days at 20ºC.

Collect potential hosts from margins of carrot crops and test for virus incidence using ELISA

Seventy-nine samples of umbelliferous weeds and crops were received for PYFV detection. PYFV was detected in eighteen samples of carrot, celery, parsnip and cow parsley but was not detected in hog weed or other weeds (Table 4). Isolates were stored and used for subsequent molecular characterisation studies.
One sample of cow parsley, identified as being PYFV positive, was found to be infested with *C. aegopodii* and a programme of single and serial acquisition and transmission experiments was undertaken to extract and isolate the viruses. The programme involved over 1000 plants using individual *C. aegopodii* and was successful in isolating AYV from PYFV and cultures of each virus have been established in suitable indicator plants.

All PCR primer combinations used amplified DNA of the expected size following RT-PCR carried out on total RNA preps from parsnip yellow fleck virus (Anthriscus strain) infected *N. benthamiana* (Figure 15). Furthermore, using primer set A 310nt of the polymerase gene of a number of isolates from carrot, cow parsley and celery was amplified by RT-PCR. In addition an AYV isolate established in chervil was amplified and the PCR products were sequenced,

aligned and a cladogram of the putative translation products developed (Figure 16). Isolates from cow parsley and carrot weakly clustered together (amino acid identities 80-100%) while isolates from celery and parsnip weakly clustered together (86-100%). The amino acid identity between these two clusters was between 86-100%. AYV clustered with the other waikaviruses (78% and 68% identity with RTSV and MCDV, respectively). The sequences show that the polymerase gene of PYFV is very variable.

Conclusions

The networks of water traps indicated that aphid abundance varied significantly with location, crop and time. In carrot crops aphids tended to be most abundant in the east Midlands and Yorkshire and least in Lancashire and east Anglia. Furthermore, numbers of non-colonising aphids species were significantly higher than the numbers of *Cavariella* spp., for example on one occasion in Shropshire *Aphis fabae*, the black bean aphid, alone contributed over 40% of the total number of aphids caught, and their role in PYFV epidemiology remains unknown. Aphids tended to be caught in carrot fields early in the season when crops are likely to be more susceptible to vector colonisation and virus transmission. The networks have proven invaluable as tools to monitor the timing and migration of aphids and the rapid response in returning results to growers has been appreciated. It is intended to utilise a similar network in the coming year.

Differences in suitability for *C. aegopodii* were observed between host plants. Parsnip appeared a more favourable host than spinach with all aphids surviving the immature stage development period. Indeed development from birth to adulthood was rapid on parsnip requiring only 15 days at 20ºC. Further studies are planned to investigate the effects of plants on aphid dynamics and extend these preliminary results to cover other potential hosts at different temperatures.

Of the samples received and tested for virus around 20% crop host and over 40% cow parsley plants were infected with PYFV. However the virus was not detected in any other weeds. These results indicate that reserviors of virus in potential host plants could be extensive and that PYFV might be more common than previously considered. Further studies are planned to investigate the role of host plants in virus epidemiology in the coming year.

The extensive single and serial acquisition and transmission experiments using individual aphids succeeded in isolating AYV from PYFV, and separate cultures of each virus have been established in appropriate indicator hosts. The extent of the success of this task has meant that the molecular characterisation studies have begun early and results have been produced sooner than expected. Analsys of the PCR products and the subsequent cladogram of the putatiuve translation products indicated that PYFV isolates from cow pasley and carrot were related but distinct from isolates from celery and parsnip which in turn were related to each other. Simialrly AYV was found to be closely related to other waikaviruses. These preliminary results indicate that the polymerase gene of PYFV is highly variable and further studies are planned to investigate this variability and characterise the virus more fully.

Technology Transfer

Close interaction with the industrial partners of the consortium and growers in general has occurred throughout the project to date; samples of aphids from the networks of water traps and suspected virus infected plants have been received from several sources and results returned and discussed with the appropriate parties. In addition several visits have been made to grower holdings and discussions regarding the project and its objectives have taken place.

Since the ELISA tools developed for routine detection of PYFV were further modified using antibodies produced in HDC project FV228 "Carrots: diagnosis of PYFV" (Spence et al., 2000) the results from the original project have been transferred to HDC members via the appropriate commodity panel activities and form a basis of introducing the project to the wider industry audience.

An article has been written for the LINK newsletter detailing the project, its objectives and potential benefits. Furthermore, preliminary results from the project have been presented at an AAB conference on virus epidemiology and used to form the basis of a poster (Boonham *et al*., 2000).

References

Boonham, N., Morton, A. Barabara, Spence, N., Barker, I., Morgan, D. 2000. Parsnip yellow fleck virus. Poster presented at the AAB Conference 'Plant Viruses', Dundee. 20-22 Spetember, 2000.

Spence, N. J., Barker, I., Mumford, R. & Wright, D.M. (2000). Carrots: diagnosis of parsnip yellow fleck virus. Final Report of project FV228, Horticultural Development Council, UK

Mix	Forward	Reverse	Size (nt)
A	7749F	8276R	537
B	7749F	8187R	438
C	7749F	8038R	289
D	7749F	8059R	310
E	8010F	8276R	266
F	8010F	8187R	177
G	8037F	8276	239
H	8037F	8187	150
I	8154F	8276	122

Table 1. Primer combinations used to amplify nucleotides of the polymerase gene

Site	Crop
Ollerton, Notts	Carrot
Harby, Notts	Parsnip
Ormskirk, Lancs	Carrot
Preston, Lancs	Celery
HRI Stockbridge, House Yorks	Carrot
Ely, Cambs	Celery
Attleborough, Nor	Parsnips
Herringswell 1, Suff	Carrot
Herringswell 2, Suff	Carrot

Table 2. Location of field sites and crops grown

Site	Crop
Market Weighton, E. Riding	Carrot
Maddock, Shrops	Carrot
SV, Notts	Carrot
Skelsmerdale, Lancs	Carrot

Table 3. Location of field and crops grown at sites provided by consortium partners

Host	Number of plants tested	Number of plants PYFV positive
Carrot	34	6
Celery	12	2
Parsnip	10	3
Cow parsley	17	7
Hog weed	4	0
Other weeds	2	0
TOTAL	79	18

Table 4. Virus incidence in potential hosts collected from margins of carrot crops

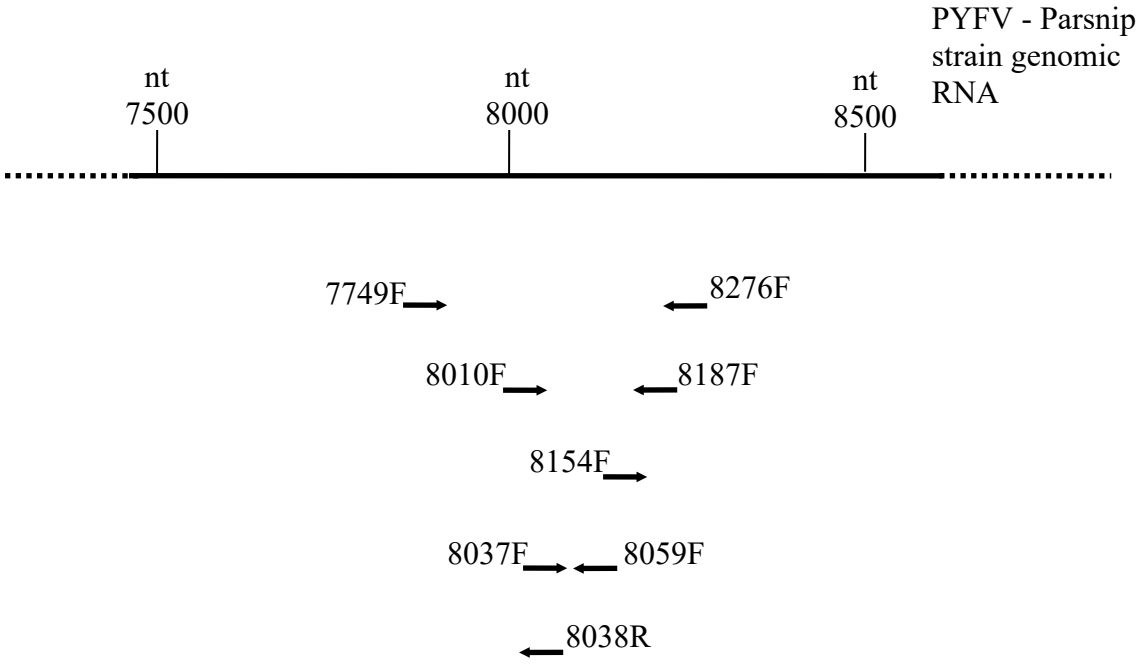

Figure 1. Nucleotide arrangment used to design degenerate PCR primers