Project title:	**A review of novel strategies to manage viruses in UK crops**
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DOCUMENT STRUCTURE

This document reviews the different methods available for virus management in various crop groups. The reader is invited to consult their specific section of interest by clicking on the hyperlink attached to each section.

The document begins with a Review Summary of the overall conclusions.

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REVIEW SUMMARY

Headline

In mitigating against the negative impacts posed by viruses on diverse agriculture and horticulture production systems, there is no one-size-fits-all approach. Instead, a paradigm shift toward deploying integrated, eco-friendly strategies is needed. Novel management practices in virus management have been identified in this review, centred around building epidemiological intelligence of virus threats, technological innovations to test for potentially new viruses and a better understanding of virus pathosystems and their interactions with the surrounding environment.

Background

Understanding the impact and management of virus symptoms in crops and /or the vectors that transmit viruses is an important issue for several AHDB sectors. This review was undertaken as a response to the need to commission new research into virus/crop management strategies.

Summary

Climate change, agricultural globalisation and international trade are driving shifts in agricultural practices and cropping systems that favour viral disease outbreaks. In the UK, growers are heavily reliant on synthetic insecticides to manage viral vectors cost effectively. However, the use of these products is coming under increasing pressure from legislation, climate change and market requirements such as reduced pesticide inputs and maximum residue levels. This, combined with insecticide resistance is having a significant impact on the both the arable and horticulture sectors. Ever advancing changes in technology provide opportunities for virus management beyond the current choices. This will allow more precise and targeted control, enabling integrated management of viruses-and their vectors-to become more of a normal practice than a concept that growers consider too much of a challenge.

This report is a review of virus management options on a national and international level that could benefit UK crop production in cereals and oilseeds, sugar beet, potato, field vegetables and protected tomato. A summary of the major virus diseases affecting these crops in the UK, their vectors and alternate hosts, their primary methods of detection and available facilities in the UK to carry out detection are provided in Tables 42 and 43.

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Recent shifts in viruses currently present in the UK

In potato, there has been a recent shift towards the *Potato virus Y* serotype PVY^N which accounted for more than 90% of PVY cases (Davie *et al.,* 2017). In vegetable Brassicas, *Broccoli necrotic yellows* (BNYV) has been recorded in the UK (Walsh, J. Pers. Comm.). In tomato, *Southern tomato virus* (STV) was first identified in the UK in April 2019, with the first case of *Tomato brown rugose virus* (ToBRFV) reported on July 12th 2019. At the time of writing, the source of this ToBRFV outbreak is currently unknown. The risk of infection to other sites remains high, especially where sites handle imported fruit.

New and emerging viruses

In 2018, a new Potyvirus infecting potatoes named *Potato yellow blotch virus* (PYBV) was identified by scientists at Science and Advice for Scottish Agriculture (SASA) (Nisbet *et al.,* 2019). In 2011, next generation sequencing identified several novel viruses in a wheat crop in Suffolk, including one that appeared in a quarter of all samples (Flint, 2015). *Wheat streak mosaic virus* (WSMV) has been detected in the UK with few issues, but strains present in other countries cause significant yield losses. The arrival of such strains in the UK may increase the importance of this virus. In carrots, there have been a number of new disease reports of viruses infecting both carrot and wild Apiaceae species, including *Arctopus echinatus associated virus* (AeaV), isolated from *Arctopus echinatus,* a perennial weed of the Apiacae family in South Africa (Richet *et al*., 2018), the first report *of Apium Virus Y* and *Carrot Thin Leaf Virus* in parsley in Slovenia (Mehle *et al*., 2019) and the first report of *Carrot torrado virus* 1 (CaTV1) and *Carrot thin leaf virus* (CTLV) from the wild Apiacae species *Torilis arvensis* ssp. *arvensis* in Greece (Lotos *et al*., 2018). Fox *et al*., 2017 discuss the newly emerging group of Nanoviruses as potentially damaging to UK pea crops: in particular *Pea necrotic yellow dwarf virus* (PNYDV) which has spread through Germany, the Netherlands and Austria (Gaafar *et al*., 2016, 2017). Ahsan and Ashfaq, 2018 demonstrated the first *Cucumber Mosaic Virus* (CMV) subgroup II infecting pea in Pakistan.

Vector biology

Resistance is now reported to most classes of insecticide (see Bass *et al.,* 2014). Neonicotinoid Resistance (Nic-R++) is only found in the southern European countries to date and confers strong resistance specifically to neonicotinoids. To date no aphids in the UK have been identified with this form of resistance. Ongoing screening programmes in the UK have also shown no evidence of resistance to pymetrozine or flonicamid in *Myzus persicae* (https://ahdb.org.uk/iraq)

Due to stringent management techniques, R3 resistance, or extreme/high levels of resistance has not existed in UK populations of *M. persicae* for the last 5 years or so, However

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glasshouse imports of overseas *M. persicae* could result in it arriving back to the UK. This is relevant as organophosphates are no longer in use anymore in the UK, except in select cases, and the range of available actives and modes of action are in steady decline.

There is currently no evidence of field resistance to insecticides in *Macrosiphum euphorbiae*, however, increased levels of carboxylesterases have been detected in laboratory experiments suggesting that there is potential for resistance to develop.

Pyrethroid resistance is becoming an increasing problem in UK pests. *Sitobian avenae* with knockdown (kdr) resistance is widespread in the UK (IRAG, 2019b).

Vectors and alternate hosts

Aftab *et al*., 2018 report the detection of PSbMV from fenugreek in Australia. Fenugreek is not yet grown in the UK, but it provides scope for other legume crops to become infected and act as alternate hosts to with PSbMV. Recent experience in the UK has suggested that *Moroccan watermelon mosaic virus* (MWMV) may be whitefly transmitted, but this is yet to be confirmed.

Detection and identification of viruses

Perdikaris *et al.,* 2011 describe a novel portable biosensor system for detection of plant viruses, designated the 'High Throughput Bioelectric Recognition Assay (BERA-HTP). This system was able to detect purified PVY, *Cucumber mosaic virus* (CMV) and *Tobacco Rattle Virus (*TRV) in single and mixed infections. In oilseed rape, Congdon *et al*., 2019 developed a LAMP assay able to detect *Turnip yellows virus* (TuYV) in leaf material and *M. persicae*. Sanchez-Navarro *et al*., 2018 report the development of a unique riboprobe named genus-probe which has the capacity to detect all members of the genus Potyvirus using a nonradioactive molecular hybridization procedure. Tiberini *et al*., 2019 developed a RT-LAMP assay for detection of *onion yellow dwarf virus* (OYDV) with the potential to be used both in laboratories and in-field. Monoclonal antibodies have been developed for *Lettuce big vein associated virus* (LBVaV, Walsh, J. Pers. Comm). To the authors' knowledge, no ELISA or other diagnostic assays are available for CaTV or *lettuce necrotic yellow virus* (LNYV).

Novel technologies

Three separate reports from 2019 (Griffel *et al.,* 2019; Moslemkhani *et al.,* 2019; Polder *et al.,* 2019) report successful detection of PVY in potato using spectral signatures using hyperspectral imaging methods for the development of a rapid and non-destructive PVY detection system. Remote sensing of WSMV using a hand-held radiometer (Workneh *et al*., 2009), satellite (Mirik *et al*., 2011, 2013) and aerial imagery (Stilwell *et al*., 2019) has been shown to be effective at discriminating WSMV infected and healthy wheat. It has been

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suggested that antiviral drugs could be used as control agents in field situations (Borodavka *et al*., 2012) and that these could be applied as nanoparticle additives to fertiliser applications (Flint, 2014). In tomato, a method based on a powerful remote sensing tool/image classification, known as hyperspectral imaging and outlier removal auxiliary classifier generative adversarial nets, havs been developed which can detect early infections of *Tomato spotted wilt virus* (TSWV, Wang *et al*., 2019). In sugar beet, work is currently being carried out in the UK to develop a virus yellows phenotyping method capable of quantifying symptoms via drone images to aid genetic mapping studies (James *et al.,* unpublished data).

Modelling and decision support systems

Ranabhat *et al.*, 2018 used modelling approaches to identify risk factors for WSMV and its vector, *Aceria tosichella*. Workneh *et al*., 2017 further developed the model to predict yield losses based on spring disease severity to assist in assessing the economic benefits of crop management decisions. The spatial dynamics of *A. tosichella* movement and virus spread were modelled by Stilwell *et al*., 2019. A number of BYDV models have been developed (Walls *et al*., 2016, Enders *et al*., 2018) and tools to optimise spray timing, targeting the second generation of aphids, based on a model using the T-sum 170 day degrees (DD) threshold are available (e.g., https://ahdb.org.uk/BYDV)

Chemical and cultural management options

Simon *et al*., 2014 showed that a permanent mesh net covering a cabbage crop significantly reduced *Brevicoryne brassicae* populations, but had no effects on *M. persicae*. This is probably explained by the larger netting size and the more globular and larger size of *B. brassicae* compared to *M. persicae*. No research has explored the potential exclusion of smaller aphids using finer nets, and whether these would affect the microclimate. In cabbage, Ngosong (2017), investigated the effects of six different pest management strategies on key insect pests of cabbage, including *B. brassicae*. Shallot planted the same time with cabbage, then sprayed with a short duration of neem had the lowest overall aphid score. In many vegetable crops, growers are investigating alternative methods of weed control between rows such as living mulches. Compost and woodchip is being evaluated in an EIP project on a Welsh organic vegetable farm for Horticulture Wales. Cucurbit growers are evaluating straw as a mulch between rows, in addition to dwarf rye to outcompete weeds in the inter-row areas. (Cook *et al*., 2019). Current research investigating the benefits of flame weeding in vegetable systems is being investigated in a European H2020 funded project IWMPraise (2016). In onions, reduced soil nitrogen in the absence of biostimulants can reduce onion thrips densities and final disease incidence without a significant loss in yield (Buckland *et al*., 2013).

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Naraghi *et al*., 2014 found that *Trichoderma harzianum* and *Talaromyces flavus* were capable of disease suppression by decreasing *P. betae* populations and promotion of sugar beet growth factors when applied as a soil treatment. Requiem, a biological-type insecticide based on the terpenoid blend QRD460 is in the registration process in the Netherlands, initially for protected crop uses, but could be looked at on outdoor crops too. With a zero residue profile, it is mainly targeting small sucking pests such as aphids and thrips. It has a relatively rapid knock-down effect, but does not persist on the leaf surface (Lacey, T., Pers. Comm.).

Applying mineral oil to potato crops has been found to not interfere with statutory growing crop inspections and is a viable method for future control of aphids. Grul'ová *et al*., 2017 and Shah *et al*., 2017 showed the potential for essential oils in controlling BYDV vectors. Such plant-based insecticides may be at lower risk of developing resistance as they often act on multiple sites (Tripathi *et al*., 2009; Rattan, 2010). Sivanto (flupyradifurone) has recently been registered in the Netherlands. This is a butenolide–a new class of insecticides, which acts on the central nervous system of insects. Although not a neonicotinoid, it could be positioned similarly in terms of rapid efficacy, quick knock-down and positive effects on virus transfer. This active has possibilities to be available in various field crops. It is likely to be limited to only one application per year per crop (Lacey, T. Pers.Comm.)

Syngenta acquired DevGen in 2013 to develop RNAi as a sprayable crop protection product to control insects; this could be a future tool for virus management, the company are also developing a biostimulant that helps elevate the effect of viruses if infection occurs. Both products are currently confidential (Newbert, M. Pers. Comm.).

Breeding and genetic tools

Hirsch *et al.,* 2014 developed Spud DB (http://potato.plantbiology.msu.edu/) for the scientific and breeding community to access the potato genome sequence and annotation datasets. Armstrong *et al.,* 2019 published a novel diagnostic resistance gene enrichment sequencing (dRenSeq) method to identify the presence of functional nucleotide binding-leucine rich repeat (NLR) genes in tetraploid potatoes. Numerous studies are available on transformed wheat and barley varieties showing resistance or tolerances to aphids (Bruce *et al*., 2015; Duan *et al.* 2018; Hou *et al.,* 2019; Cejnar *et al.*, 2018). Kis *et al*., 2019 demonstrated the potential for CRISPR to develop WDV resistance in barley. In oilseed rape, it is proposed by Wang *et al*., 2011 and Mulot *et al*., 2018 that RNAi-mediated virus control could be delivered as a foliar spray.

In peas, Kaur *et al*., 2019 describe a method used to eliminate *bean yellow mosaic virus* (BYMV) from an infected gladiolus crop *in vitro,* using cormel explants which were subjected to thermotherapy, chemotherapy and electrotherapy. To date, advances in developing

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transgenic pulse crops have primarily been limited to laboratory trials and have not been commercialized at the large scale. Genomic resources, such as bacterial artificial chromosome (BAC) libraries, are available for various pulse crops including peas and beans (see Yu, 2012 and Meziadi *et al*., 2017 for reviews).

In cabbage, Ma *et al*., 2019 showed that CRISPR/Cas9 can be used to efficiently mutate genes of interest.

Agrobacterium mediated transformation has been accomplished in all of the three Cucurbitaceae genera with regeneration performed through shoot organogenesis, however transformation efficiency has been very genotype dependant (Klocke *et al*., 2010; Manamohan *et al*., 2011). Virus resistance of cucumber plants has been investigated using Cas9/subgenomic RNA (sgRNA) to disrupt the function of the recessive eIF4E gene at two sites (Chandrasekaran *et al*., 2016).

Walley *et al*., 2017 describe the publicly available UK Vegetable Genetic Improvement Network lettuce diversity set. This population is accompanied by a panel of breeder-friendly lettuce-specific KAS markers that have been anchored in the *Lactuca sativa* genome assembly.

Npthnagel *et al*., 2017 evaluated a range of cultivars, gene bank accessions and breeding lines of asparagus as well as thirty-four accessions of wild relatives of Asparagus for resistance to *Asparagus virus 1* (AV-1).

Fan *et al*., 2014, 2015 and Jafarzade *et al.,* 2019 have demonstrated the use of deep sequencing transcriptomics to investigate the responses of *Nicotiana benthamiana* and *Beta macrocarpa* to infection with *Beet necrotic yellow vein virus* (BNYVV) infections. Jiang *et al.,* 2019 has developed a BNYVV infectious cDNA clone and engineered a set of BNYVV-based gene expression vectors that can express recombinant proteins in *N. benthamiana* and sugar beet. These vectors can be used to investigate the subcellular co-localisation and function of multiple proteins in tissues of systemically infected plants. They also demonstrated that BNYVV-based vectors can be used to deliver guide RNA for CRISPR/Cas 9 plant genome editing.

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Table 3. A prioritised list of control options, both applied and fundamental which should be investigated and/or applied to UK production systems

1. Monitoring and thresholds	
Crop	**Method**
All crops	Knowledge exchange to inform growers and advisors on how best to detect for viruses and avoid misdiagnosis.
	Development of health and safety and legislation frameworks for drone usage.
	Investigate modelling of insecticide resistance development due to loss or gain of individual modes of action.
	Monitor for emerging cases of resistance in UK aphid species covering changes in resistance levels and new cases of resistance.
	Monitor for arrival of non-indigenous aphid species vectoring new viruses or virus strains.
	Develop further NGS methods for identification of new and unknown viruses outside the scope of those already being investigated.
	Further develop hyperspectral imaging methods to identify viral infection
Sugar beet	Development of a high through put in field bioassay for virus yellows detection using a Bioelectric Recognition Assay (BERA-HTP) or LAMP assay. Development of high through-put qRT-PCR methods for persistent and semi-persistent yellows viruses e.g. field collected aphids, host canopy and tap roots to understand proportion of aphids carrying the individual viruses and geographical spread within crops. Update pesticide thresholds for control of virus yellows and develop improved decision support systems (DSS).
Cereals & oilseed rape	Improve monitoring methods for BYDV and TuYV vectors. This should include schemes that determine the proportion of vectors carrying the virus, remote sensing, image analysis for aphid identification and novel molecular diagnostics.
Cereals	Develop improved decision support systems (DSS) for the management of BYDV. This should include understanding of the impact of the proportion of aphids carrying BYDV on yield and management decisions.
Potato	Update predictive models so they are capable of predicting aphid migration and virus risk in a changing environment.
	Deployment of portable diagnostic devices such as LAMP assay as a first line of diagnostic.
Oilseed rape	Develop models to better predict infection, spread and yield impact of TuYV. These should then be developed into DSS to assist in management of the virus.

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Root crops	Perform baseline surveys on the prevalence of PYFV, CYLV and CaTV in the UK.
	Further investigate the relationship between numbers of willow-carrot aphid trapped and the amount of damage to the crop if left untreated.
	Establish the potential for carrot viruses to be seed transmitted.
Peas	Expand on the number of viruses which can be tested for through commercial seed testing services.
	Further develop molecular assays for detection of pea specific viruses.
	Monitor for the presence of the newly emerging group of Nanoviruses in the UK, particularly PNYDV.
	Adapt and develop the model used for forecasting PSbMV in Australia for use under UK climatic conditions.
Vegetable Brassicas	Knowledge exchange activities to improve grower and advisor awareness around monitoring for virus symptoms before heads go into storage.
	Explore the potential for using epidemiological modelling of TuVY in vegetable Brassicas.
Lettuce	Establish which pathotypes of LMV are present in LMV infected lettuce in the UK.
	Further investigate possible additional vectors and alternative hosts for LNYV.
	Develop immuno-based assays for LNYV, if none commercially available already.
Alliums	Develop a monitoring service for OYDV and IYSV which both have the potential to become established in Allium crops such as onion and leek in the UK
Asparagus	Develop a seed testing service for AV-2 so that seed can be virus-indexed before purchase,
	Further develop decision support systems and prediction modelling for aphid control in minor use crops such as asparagus.
Tomatoes	Improved ToBRFV detection, including a rapid species specific diagnostic test e.g. LFD kit, indicator plants etc.
	Knowledge exchange of the symptoms of emerging viruses, including ToBRFV to all members of the tomato industry, especially crop workers.
2. Cultural controls and hygiene	
All crops	Increased research into disruptive rotational control of aphids – by species, variety and chemistry
	Investigate alternative cultural control methods such as mineral oil application and crop borders to prevent virus spread.

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Peas	Establish the potential for new legume crops being introduced into the UK to act as potential sources of new viruses and alternate hosts of current pea viruses.
Field vegetables	Evaluate the potential for use of mulches (recyclable plastic, woodchip or straw) as control method for weeds as alternate, and the effect on the crop.
Vegetable Brassicas	Explore the potential of using different types of netting to exclude small and larger aphids, and its impact financially as well as its effect on the microclimate of the crop.
	Investigate the use of intercropping with catch crops such as shallots for management of cabbage aphids.
Cucurbits	Establish the routes e.g. through plant propagators, alternative trade pathways through which Cucurbit viruses enter the UK.
	Establish and enforce better plant health and quarantine standard to prevent new viruses of Cucurbits entering the UK.
3. Varietal resistance	
All crops	Review the ethics and regulations surrounding the use of gene editing and CRISPR technology in the UK.
Cereals	Develop varietal resistance/tolerance to BYDV in wheat.
Sugar beet	Continued development of durable host resistance/tolerance to the virus yellows complex (BYV, BMYV, BChV). Development of varieties with host resistance to *Myzus persicae* Identification of novel BNYVV resistance sources to complement Rz1/Rz2 technology to provide protection against resistant breaking strains.
Field vegetables	Examine the potential for resistant varieties developed abroad to be grown in under UK climatic conditions.
Vegetable Brassicas	Determine the resistance/tolerance status of currently available vegetable Brassica varieties through creation of independent trials data.
Tomato	Development of resistant varieties to ToBRFV, PepMV, STV, ToCV, TICV and those resistant to the new SW5 resistance breaking strain of TSWV.
Cucurbits	Exploit sources of genetic resistance and tolerance already available in other Cucurbit spp. and their feasibility for transfer into commercially grown courgette.
4. Vector and viral biology	
All crops	Inform growers on all potential routes for aphid infestation and issue guidance for control
Sugar beet	Updating research on the risk of virus yellows spread via alternative hosts (weeds, wild and cultivated beet including fodder, red beet, energy beet) across the UK, either by acting as a viral pool or by encouraging overwintering of aphid vectors.

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Cereals and oilseed rape	Improved understanding of how aphids locate crops and whether this can be used in BYDV and TuYV management.
Carrot	Establish the relationship of the association of symptoms with CMD infection
	Investigate the biological properties of CaTV to allow for the development of immuno-based diagnostic assays.
Cucurbits	Investigate the potential for MWMV to be whitefly transmitted
Tomato	Establish the longevity of ToBRFV on different surfaces and within the endosperm and if it is spread via the irrigation system/nutrient feed solution.
	Identify alternative host species of ToBRFV and other viral issues
	Further research into STV to establish pathogenicity, or the requirement for mixed infections for symptom development.
5. Biological and alternative control methods	
All crops	Research and KE activities into habitat management to improve natural enemy activity.
	Further investigate the potential of biopesticides for control of aphid vectors under field conditions.
Cereals and oilseed rape	Develop a better understanding of the impact of natural enemies have on controlling BYDV and TuYV vectors.
Tomato	Development of mild viral strains, similar to the PepMV®-01, to vaccinate against ToBRFV.
	Identify new/existing WFT and whitefly controls (chemical/biological or technological) for use in protected tomato
6. Chemical control	
All crops	Investigate the use of adjuvants or other substances to improve efficacy of currently available insecticides and increase crop safety.
	Development of novel active ingredients which can deliver speed of knockdown for controlling transmission of semi-persistent viruses.
	Investigate improved resistance management strategies for insecticide resistance in key virus vectors.
Field vegetables	Investigate how market driven perfection of products drives the use of insecticides as control options and how this could be changed to increase the use of IPM
Vegetable Brassicas	Continue to evaluate new actives which are being developed for oilseed rape in vegetable Brassicas to widen the range of actives available.
Tomato	Identify effective disinfectants, on a selection of different surfaces, for effectively eliminating ToBRFV.

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Recommendations.

- ***Improve awareness of potential viral threats:***

The review identified a large number of potential viruses which could threaten crops in the UK in the future. Factors such as climate change and importing of plants from outside of the UK could result in these viruses becoming established in the UK, for which the DEFRA Plant Health Risk Register provides information. Additionally, communication with advisors and growers suggest that problems in crops may be misdiagnosed because of a lack of awareness that such a broad range of viruses can infect their crop of interest, for example, virus symtoms in lettuce can often be mistaken for oedema. More knowledge exchange activities centred on improving awareness of the range of viral threats which exist, and how to recognise them would improve this.

- ***Increase access to, and the use of, current knowledge:***

There is more information on virus management and vector control known than is readily available to advisors and growers. Available research is often contained in academic publications which are either not readily available to the non-science community and/or is not easily translatable to practical information. Using current knowledge better is a very high priority. For management of vectors which affect multiple crops such as *M. persicae*, a knowledge hub that is irrespective of crop sectors should be developed to provide simple messages for growers and advisors. Put in comment about academic

- ***Reduce the gaps between practical knowledge and fundamental research:***

There is too great a gap between applied knowledge generated for growers and fundamental research at an academic level. More knowledge exchange activities including hands on workshops, crop walks and open days e.g. on Strategic Farms will ensure fundamental research translates into tangible changes in practices.

- ***Maximise pesticide availability:***

Good stewardship of current active substances is vital. Companies, regulators and users need to work together to develop new actives and retain old actives through establishing and supporting best practice guidelines.

- ***Develop a better understanding of application and management of biopesticides:***

As the use of biopesticides increases, it is important that biopesticides are not used as a simple like-for-like replacement for conventional chemical pesticides. Further work and

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knowledge exchange activities on the use of biopesticides in field cropping systems is required.

- ***Agree funding for an integrated approach to virus management:***

There is a strong social and political desire to maximise non-chemical and more integrated approaches. Government and industry need to working together to achieve this.

- ***Research into management of viruses needs to be considered more strategically:***

Overall, the current approach to virus management is very often based on specific viruses and/or in specific crops. A more strategic approach to virus management over the whole cropping system is needed, particularly for agnostic insect vectors such as *M. persicae* and *B. tabaci* and virus species such as CMV and TuYV which affect multiple crops in arable and horticulture. This approach should cover the four basic principles of virus management;

- Start with clean, virus free material
- Grow in the absence of vectors and alternate hosts
- Crop rotations and isolating from similar crops
- Exploit available varietal resistance and tolerance

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REVIEW

Introduction

In the UK, growers are heavily reliant on synthetic insecticides to cost effectively manage viral vectors. However, the use of these products is coming under increasing pressure from legislation, climate change and market requirements such as reduced pesticide inputs and changes to maximum residue levels. This, combined with insecticide resistance is having a significantly negative impact on arable and horticulture sectors. Pesticide regulation such as the review of Approval for Active Substances, Maximum Residue Limits (MRLs), Definition of Endocrine Disruptors, Sustainable Use Directive (SUD), Water Framework Directive (WFD) and Candidates for Substitution, continue to erode the number of available active substances. This, combined with the development of resistance to key active ingredients important for the control of aphid species such as *Myzus persicae* is having a significant negative impact on the horticulture sector but is also affecting potatoes, cereals & oilseeds and sugar beet.

This review will focus on key viruses that the arable and horticulture sectors face in the UK, the consequences of limited chemistry to control vectors and the new and emerging technology that will help relieve future pressures on the industry.

Potatoes

Potato is the second most economically important crop grown in the UK after wheat. Potato viruses are a major concern to the UK potato industry with estimated associated losses of between £12M and £22M per annum. At present there are over 40 known viruses that affect potato globally. Many potato viruses are transmitted via seed; once a tuber has become infected any progeny generations from that tuber will also be infected and will provide the main source of inoculum. Potato viruses are transmitted in nature by a large variety of microorganisms, fungi, protist and invertebrate vectors including nematodes and several insect species and, most importantly, aphids.

Cereals

The impact of viruses on cereals is of major concern, especially in light of reductions in the availability of chemical control options and insecticide resistance. There are many viruses affecting cereals, and for this reason this review will focus on those infecting wheat and barley. The most important in the UK are *Barley yellow dwarf virus* (BYDV) and *Soil-borne cereal mosaic virus* (SBCMV). The latter is well controlled by the use of resistant varieties while the former has been well managed for several years through a combination of neonicotinoid seed treatments, foliar insecticides and cultural control. However, recent restrictions mean that

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neonicotinoid seed treatments are no longer available for use, making control of BYDV much more difficult.

Oilseed rape

Oilseed rape (OSR) (*Brassica napus* L.) is a highly important break crop in the UK but the area sown has reduced in recent years. Poor pest control is a key reason for this reduction. The loss of neonicotinoid seed treatments and the increasing issue of insecticide resistance has reduced pest control options and substantially increased the risk of yield reductions and crop failure. However, markets are limited for alternative break crops meaning that OSR remains the preferable break crop for many growers.

The impact of viruses on OSR is of major concern to the industry, especially with reductions in the availability of chemical control options in recent years. There are several OSR-infecting viruses in the UK with Turnip yellows virus (TuYV) having the greatest impact in terms of the proportion of crops affected and yield reductions. On-farm OSR yields have barely increased since 1985, with the long-term average being 3.1 t/ha in comparison to an estimated yield potential of greater than 6.5 t/ha. It has been suggested that TuYV is a primary reason for OSR not reaching its full genetic yield potential in the UK.

Field vegetables

With a decreasing number of pesticides available to the horticultural industry, management of viruses and their alternate hosts and vectors continues to be more challenging across many horticultural crops. Losses caused by viruses can be high; in head Brassicas, yield losses from virus and damage to small plants can be as much as 30% and in many systems, such as cucurbits, the whole crop can be lost. Indeed, a gap analysis conducted for AHDB Horticulture in 2016 identified virus control as a high priority in asparagus, cucurbits and head Brassicas, with management of aphids a high priority in 17 out of 21 key field vegetable crops grown in the UK.

The loss of key active ingredients will destroy resistance management strategies and make control of aphids such as *Myzus persicae* impossible, substantially increasing the risk of yield reduction and crop failure. It is essential that these crops be kept virus free, particularly for long season crops such as overwintered cabbage, Brussels sprouts and perennial crops such as asparagus. These crops are exposed to infection for a longer period of time and there is greater opportunity for the virus to affect tonnage and quality because of the long growing period. Poor control also has a knock-on effect as it increases the activity of predators and their likelihood as a contaminant at harvest

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Protected tomato

Tomato is one of the highest value crops grown in the UK, with 65,000 tonnes of fruit produced in over 170 ha of protected glass. Growing in protected structures enables precise crop steering, maximising fruit quality and yields, whilst limiting pest and disease issues which may occur in the field. However, pest and disease issues can rapidly develop in these densely populated areas, placing the crop at risk.

Over 100 viruses naturally infect tomato (*Solanum lycopersicum*), with more demonstrated to infect via artificial inoculation studies. Symptoms differ by virus species and strain, and individual strains will exhibit differences in symptom expression and severity. Tomato plants can also become infected by multiple viruses, which can exacerbate symptoms. Tomato viruses can affect all parts of the host plant; the roots, stems, foliage and fruit. Infection usually leads to a reduction in fruit quality and/or fruit number, impacting the quantity of marketable fruit, leading to economic losses.

Sugar beet

The EU is the world's leading beet sugar producer, producing approximately 50% of global sugar beet volume, and contributing around a fifth of the global sugar production. Almost 18 million tonnes of beet sugar are produced in the Union each year, with approximately 3.3 million ha of sugar beet grown in 2017. In 2018, the European Commission enforced a ban on the three main neonicotinoids (clothianidin, imadacloprid and thiamethoxam) for all outdoor uses, due to mounting evidence that they are highly damaging to pollinators such as bees. The 2019 season will see the first crop grown without neonicotinoid seed treatments since the early 1990's, leaving the industry extremely vulnerable to the return of highly damaging viruses, such as the *Virus Yellows complex*, carried by *Myzus persicae*.

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Materials and methods

A literature search was carried out to ascertain knowledge on virus management in 12 different crops/crop groups. Literature searches were carried out principally using Google Scholar, Web of Science, Science Direct, NCBI PubMed and the AHDB Horticulture website. In addition to formally published scientific literature, conference proceedings, PhD theses and project reports were consulted. A number of agronomists and scientists were also consulted informally to obtain real-time, current information on virus management in each individual crop group. In-house expertise were available to help direct searches towards relevant information. The review includes 'text book' information on individual viruses as well as recent knowledge gains with references to allow readers to seek greater depth of interest to them. Where knowledge is lacking, this has been highlighted. Recent papers, from 2000 onwards, or when the last review for individual viruses was conducted have been the primary focus. However, where deemed necessary and relevant, older literature has been explored, such as when limited recent work has been carried out on certain pathosystems. Relevant AHDB Horticulture reports were assessed to highlight new information and where gaps still exist in order to identify where further research or development would be worthwhile. After the review section, information from all sources has been used to produce a table with key knowledge areas and gaps for each crop group/type.

The following people kindly edited and/or provided feedback for the review:

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Potato: Dr Christophe Lacomme, SASA, Dr Max Newbert (Syngenta)

Oilseed rape: Luke Cotton, Prof John Walsh (Warwick University)

Field vegetables: Matt Kettlewell (Procam), Dr Tim Lacey (Bayer), Prof. John Walsh (Warwick University), Dr Max Newbert (Syngenta)

Protected Tomato: Dr Tim O' Neill, Derek Hargreaves, Dr Phil Morley (APS Salads)

Sugar beet: Dr Mark Stevens, BBRO, Ian Munnery (Sesvanderhave)

Virus management in potato

Potato (*Solanum tuberosum*) is grown by over 130 countries worldwide, and is considered the fourth most important staple food source after rice (*Oryza sativa*), maize (*Zea mays*), and wheat (*Triticum aestivum*) (Ezekiel, *et al.,* 2013; Zhang *et al*., 2016). Globally, ~388,190,674 tonnes of potatoes were produced from ~19,302,642 ha of land in 2017 (FAOSTAT), and they are consumed by over one billion people. China produces the greatest percentage of the global potato crop at 24%, but North America has the highest yields in the world. The UK is the 12th largest potato growing country and one of the highest consumers at 90kg per person per annum. The British potato industry contributes approximately £731 million to the economy

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at leaving the farm gate and up to £5.7 billion at the consumer level (BPC, Market intelligence report 2012-13). It is the second most economically important crop grown in the UK after wheat.

Potato viruses are a major concern to the UK potato industry with estimated associated losses of between £12M and £22M per annum (Twining *et al*., 2009). At present there are over 40 known viruses that affect potato globally. For many potato viruses once a tuber has become infected, any progeny generations from that tuber will also be infected and will provide the main source of inoculum, known as seed transmission. The other method for transmission is via a vector. Potato viruses are transmitted in nature by a large variety of microorganisms, fungi, protist and invertebrate vectors including nematodes and several insect species - most importantly, aphids. For the purposes of this review, we will be primarily focusing on viruses relevant to the UK, such as *Potato Leaf Roll Virus* (PLRV), PVY, PVA, PVX, PVV, PVS, *Potato Mop Top Virus* (PMTV) (vector powdery scab) and *Tobacco Rattle Virus* (TRV).

Current viruses present in the UK

More than 40 viruses are known to infect potato from several different genera, including the Poleroviruses, Potyviruses, Potexviruses, Carlaviruses and Tobraviruses. Within this review we will be focusing on potato viruses found in the UK, paying special attention to *Potato Virus Y* (PVY). The prevalence of PVY in the UK was highlighted in 2009, when Pickup *et al.* published epidemiology data showing that PVY was the cause of 35% of virus symptoms of Scottish seed potatoes between 1998 and 2008. *Potato virus A* (PVA) was the second most prevalent virus at 22% of positive laboratory diagnoses. *Potato Leaf Roll Virus* was present at a level of 14% and *Potato Virus V* (PVV) accounted for 4% of the total virus. Aphid transmissible viruses were therefore responsible for over 75% of the virus recorded from Scottish seed crops over this period. Major viral threats to seed and ware potato crops, past and present in the UK, can be found in Table 1.

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Table 1. Summary of potato viruses in the UK. Present viruses are those which are known to be currently present in UK crops.

Virus	Acronym	Mode of Transmission	Genus	Reference
Potato Virus Y	PYY	Aphid, Mechanical	*Potyvirus*	Beemster & de Bokx, 1987
Potato Leaf Roll Virus	PLRV	Aphid	*Polerovirus*	Beemster & de Bokx, 1987
Potato Virus A	PVA	Aphid & Mechanical	*Potyvirus*	Maclachlan *et al*., 1953; Ahmadvand, 2012
Potato Mop Top Virus	PMTV	Soil borne pathogen	*Pomovirus*	Calvert, 1968; Fribourg & Nakashima, 1984
Potato Virus X	PVX	Mechanical	*Potexvirus*	Silva *et al.* 2005
Potato Virus V	PVV	Aphid	*Potyvirus*	Rozendaal *et al*., 1971
Tobacco Rattle Virus	TRV	Nematode	*Tobravirus*	Robinson and Harrison, 1989
Potato Virus M	PVM	Mechanical & Aphid	*Carlavirus*	Smith, 1972
Potato Virus S	PVS	Mechanical & Aphid	*Carlavirus*	Harrison, 1971; Brien, 1976; Rich, 1983
Tomato Black Ring Virus	TBVR	Nematodes	*Nepovirus*	N/A

Potato virus Y

Potato virus Y (PVY, family: *Potyviridae*, genus: *Potyvirus*) is globally the most economically important virus to affect cultivated potato, both in terms of yield (losses of up to 80%) and in quality (Valkonen, 2007). According to recent estimations, PVY is able to affect up to 50% of potato crops in China, which is the world's largest potato producer (Wang et al., 2011). In other parts of the world, average incidences of PVY are around 44% in USA (Gray et al., 2010), nearly 40% in Poland (Hasiów-Jaroszewska et al., 2014), 37% in Kenya (Were et al., 2013), 34% in Canada (Gray et al., 2010), and 16.5% in Ireland (Hutton et al., 2015).It has an extremely large host range which includes cultivated solanaceous species (potato, tobacco, tomato, pepper, petunia) and many solanaceous and non-solanaceous weeds.

PVY strains can be classified into seven distinct strain groups: PVY^{O} (ordinary or common strain), PVY^{N} (necrotic strain), PVY^{C} (stipple streak strain causing leaf drop of potato), PVY^{Z}, PVY^{E}, $PVY^{N\text{-}Wi}$ (N-Wilga) and PVY^{NTN} (n-Tuber Necrosis) which can be further divided into European ($PVY^{EU\text{-}NTN}$) and North American isolates ($PVY^{NA\text{-}NTN}$) (reviewed by Singh, 2008). Additional diagnostic methods such as ELISA, genome sequencing, recombination analysis

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and phylogenetic studies have also highlighted the high inter-strain diversity of PVY (Singh *et al*., 2008; Kerlan *et al*., 2011; Karasev and Gray, 2013).

Field surveys worldwide have identified that PVY is generally increasing and recombinant strains and variants are displacing previously non-recombinant strains such as PVY^{O} and PVY^{N} (Crosslin *et al.,* 2002; Gray *et al.,* 2010; Rigotti *et al.,* 2011; Krasov and Gray, 2013; Elwan *et al.,* 2017). It is thought that the milder symptoms expressed by recombinant strains such as PVY^{NTN} and $PYV^{N\text{-}Wi}$ contribute to the spread of the isolates, as more symptomatic strains such as PVY^{O} are removed from the crop, preventing the virus from being passed on in the seed (Gray *et al.,* 2010; Karasev and Gray, 2013).

While virus incidence is currently low in Scottish seed potato crops, PVY has become the most prevalent virus. A long-term study monitoring PVY^{N} and PVY^{O} serotypes from 1993 to 2015 revealed a recent shift towards PVY^{N} which accounted for more than 90% of PVY cases. (Davie *et al.,* 2017). Molecular characterisation of the isolates indicated that 80-90% belonged to the recombinant European (EU)-NTN group, with North-American (NA)-NTN and non-recombinant EU-NTN variants accounting for the remainder. In addition, surveys of a range of PVY isolates (representing the main strain and phylogenetic groups) suggest that PVY has the ability to overcome hypersensitive response-mediated resistance, with significant differences between isolates of the same group. In contrast, extreme resistance genes (Ry_{adg}, Ry_{sto}) provide efficient resistance to PVY transmission to progeny tubers. The isolate found to have the highest transmission rate was recombinant $PVY^{EU\text{-}NTN}$, when compared to the transmission rate of PVY^{O} and $PVY^{NA\text{-}NTN}$. The authors concluded that the $PVY^{EU\text{-}NTN}$ isolate may have a competitive advantage over the PVY^{O} and $PVY^{NA\text{-}NTN}$, however the exact mechanism is still to be fully established (Davie *et al.,* 2017).

Potato Leaf Roll Virus (PLRV)

Potato leaf roll virus (PLRV, family: *Luteoviridae*, genus: *Polerovirus*), also known as potato phloem necrosis, is the most damaging and widespread virus of potato after PVY (Smith, 1972). It can cause large yield losses of up to 90% and can reduce tuber quality due to necrosis in some varieties (Khurana, 2004). However, the incidence of PLRV remains low when seed stocks are frequently exchanged and systemic insecticides are used to control aphid vectors. Primary infection can cause erect young leaves with chlorosis, while secondary symptoms include stunting of the shoots and upward rolling of mature leaves, which turn chlorotic, leathery and brittle (Khurana, 2004). Examination of genomic PLRV sequences have shown that genetic diversity is relatively low, however sequence differences in open reading frame 0 can help distinguish Australian and Peruvian isolates from those identified in Europe (Guyader and Ducray, 2002). The apparent low mutation fixation rate seems to be

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unique to PLRV compared to other poleroviruses. PLRV has the potential to become even more of a threat in potatoes with less neonicitonioid pesticides available; mineral oils do not help as much with PLRV due to it being persistent, unlike PVY which can be lost from the aphid as it perturbed from feeding (Newbert, M. Pers Comm).

Potato Virus X (PVX)

Isolates of *Potato virus X* (PVX, family: *Alphaflexiviridae*, genus: *Potexvirus*), are found globally in potato-growing regions, infecting a wide range of hosts, particularly the *Solanaceae* family. PVX is usually a moderately pathogenic virus when it infects alone, but it can cause significant economic loss in synergistic co-infection with some potyviruses, especially *Potato Virus Y* (Khurana and Singh, 1988). PVX strains can be classified into four groups (X1, X2, X3, X4) according to their reactions to localized hypersensitivity to *Nb* and *Nx* resistance genes and extreme resistance *Rx* (Santa Cruz and Baulcombe, 1995). Group 1 (X1) causes a hypersensitive response in the presence of *Nb* and *Nx*. Group 2 (X2) causes a hypersensitive response to only *Nb*. Group 3 (X3), only with *Nx*, and group 4 (X4) does not exhibit a hypersensitive response to either and is unable to infect plants carrying an *Rx* gene (Huang, 2010). There is no evidence for recombination between different PVX strains in nature.

Potato Virus A (PVA)

Potato Virus A (PVA, family: *Potyviridae*, genus: *Potyvirus*) is widely distributed in the potato growing areas of Europe and North America. PVA is limited to the family *Solanaceae* and its main plant species host is potato. PVA can decrease potato yield by up to 40% in synergistic infections with *Potato virus X* (PVX) or *Potato virus Y* (PVY) (Hooker, 1981). Symptoms vary from mild mosaic to rugosity of the leaves. Valkonen *et al.,* 1995 studied three different strain groups of PVA, PVA-U, PVA-M and PVA-B11 collected in Michigan, Maine and Hungary, respectively. The strain groups were eventually named PVA-1, PVA-2 and PVA-3 and can be distinguished from each other by coat protein sequence and different abilities to infect potato systemically (Valkonen *et al.,* 1995; Rajamäki *et al.,* 1998). PVA strain group 1 includes isolates eliciting the hypersensitivity gene *Na*, PVA strain group 2 include isolates which are able to infect King Edward without triggering the hypersensitive response and PVA strain group 3 includes isolates that elicit no hypersensitive response in potato cultivars carrying the *Na* or $Na_{KE.}$ Since the identification of PVA strain groups, there has been limited research carried out with regards to understanding PVA diversity inside and outside of Europe.

Potato Virus V (PVV)

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Potato Virus V (PVV, family: *Potyviridae*, genus: *Potyvirus*) is an aphid transmitted, non-persistent virus which was first reported in the Netherlands in 1971 (Rozendaal *et al*., 1971), as a diverging strain of *Potato virus Y* (Y^C – G1). It has now been reported in potato cultivars in the Andean region of South America and parts of Europe, including Netherlands, Finland, Scotland, Norway and Sweden and is known to cause significant damage, particularly in combination with viruses such as PVY and PVA. No individual strains have been reported to date.

Potato Mop Top Virus (PMTV)

Potato MopTop Virus (PMTV) (family: *Virgaviridae*; genus: *Pomovirus*) affects tuber quality and reduces potato yield and can be found in North and South America, Asia, northern and central Europe and some parts of Africa (Domfeh *et al.* 2015). The vector for PMTV is *Spongospora subterranea* f.sp. *subterranea* (Sss) a soil borne pathogen, which also causes powdery scab. Typical symptoms caused by PMTV are raised lines or slightly raised brown or rust coloured lines or rings on the surface of potato tubers or arcs; these primary infection symptoms are known as spraing symptoms in the flesh of the potato tubers. Tubers with secondary infection exhibit deep cracks or reticulations, or freckled or blotchy surface markings (elephant hide blemishes) or distortions (Harrison and Jones, 1971). The same symptoms are caused by the *Tobacco Rattle Virus* (TRV), both of which make the tubers unacceptable for consumption or processing. Mild foliar symptoms can consist of yellow blotches, rings and chevrons on the leaves and severe infections can lead to shortening of internodes, resulting in a dwarfed appearance (Calvert, 1968). Foliar symptoms are greatly affected by environmental conditions, such as temperature, light and rainfall.

The origin centre of PMTV is the Andean region of South America (Tenorio *et al.,* 2006) and the virus spread to the United States and Canada. Later PMTV was detected in potato tubers of England, Scotland and Ireland (Calvert and Harrison, 1966). PMTV has been detected in the Czech Republic and Switzerland (Schwärzel, 2002). More recently, PMTV was detected in Poland (Budziszewska *et al.,* 2010), the Hokkaido islands of Japan (Nakayama *et al.,* 2010), in China and in Nordic countries such as Norway, Sweden, Denmark and Finland (Santala *et al.,* 2010). A number of isolates collected in Denmark, Scotland, Sweden, the Czech Republic and Canada have been sequenced to identify sequence variation and possible emerging strains. Analysis of the sequences showed that overall the PMTV genome is largely conserved (Cerovska *et al.,* 2003; Mayo *et al.,* 1996; Nielsen and Nicolaisen, 2003; Pečenkova *et al.,* 2004; Sandgren *et al.,* 2001).

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Tobacco Rattle Virus (TRV)

Corky ringspot disease (CRS) or spraing of potato is caused by the *Tobacco Rattle Virus* (TRV, family: *Virgaviridae* genus: *Tobravirus*) and is transmitted by trichodorid nematodes (Robinson and Harrison, 1989). The virus can be found world-wide (Brunt et al. 1996) but generally the disease in potato can be found in sandy soils where the nematode vectors thrive. Symptoms include necrotic arcs, concentric rings or diffuse brown spots in tuber flesh, stem-mottle (distortion, stunting and mottling) and aucuba (yellow spots) in the foliage. Spraing symptoms can also be caused by infection by *Potato Mop Top Virus* (PMTV), which is transmitted by the powdery scab pathogen. It is not possible to distinguish between TRV-induced spraing symptoms and those caused by PMTV. Blemished tubers are unmarketable and the virus is considered a serious threat to potato quality in European countries and in the US (Weingartner and Shumaker, 1990). TRV exists as different isolates which can be categorised as either Mulitplying (M) or Non-multiplying (NM) types. Non-multipying isolates lack the genetic components to produce the coat protein and consequently means that NM isolates cannot be transmitted by the vector nematode. However, NM isolates can still replicate and spread from cell to cell within the plant and can produce characteristic spraing symptoms. Both serotypes can co-exist in fields.

New emerging viruses

Potato Yellow Blotch Virus (PVBV)

In 2018, a new species of the genus Potyvirus infecting potatoes, was identified by scientists working at Science and Advice for Scottish Agriculture (SASA) in Edinburgh. The newly identified virus was discovered in a breeding line 99m-022-026 in Scotland (Nisbet *et al.,* 2019) and subsequently named '*potato yellow blotch virus'* (PYBV). The infected plants show isolated yellow blotches on the leaves. The unusual yellow symptoms on the foliage could have been mistaken for other potato-infecting viruses (such as *potato aucuba mosaic virus*, *potato mop virus* or *tobacco rattle virus*). Sequence analysis shows that PYBV is closely related to *Potato Virus A* (PVA), with an overall 72% identity at the nucleotide level for the whole genome (Nisbet *et al.,* 2019). The host range of PYBV was found to be comparable to PVA on solanaceaous and non-solanaceous indicator plant species with the exception of *Solanum demissum* A and Y. Different symptoms were also observed for PYBV and PVA in *Nicotiana benthamiana, Nicotiana hesperis* and *Nicotiana occidentalis* P1. The susceptibility of potato (*Solanum tuberosum*) cultivars to PYBV and PVA was also similar (Nisbet *et al*., 2019). In over 5 years of investigation, PYBV has not been found in commercial seed and ware potato crops in Scotland, so it is considered rare. However, further studies are

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required to fully understand the epidemiology of PYBV, in order to understand its natural hosts, incidence in crops and transmission efficiency by insects.

Transmission and vectors of key viruses

Potato viruses are transmitted by a number of vectors. Whilst the majority of virus infections seen globally are transmitted by aphids there are other viruses which are transmitted by nematodes or fungi or physical contact. Aphid transmitted potato viruses may be transmitted in a persistent (e.g. PLRV) or a non-persistent manner (e.g. *Potato Viruses Y, A* and *V*). Persistently transmitted potato viruses infect the vector aphid for its lifetime and any plants on which an aphid then feeds will be at risk of acquiring the virus. Non-persistently transmitted potato viruses can only be transmitted immediately after aphids have fed on an infected plant. Non-colonising aphid species, such as cereal aphids, that do not use potato as a host but alight on potato plants and probe the leaves, can also transmit these viruses. Vectors of the key potato viruses predominantly found in the UK can be found in Table 2.

Table 2. Vectors of the key UK potato viruses

Virus	**Vector**
Potato Virus Y	Aphid transmission e.g. *Myzus persicae, Acrthosiphon pisum, Rholpalosiphum padi* etc.
Potato Leaf Roll Virus	Aphid transmission e.g. *Myzus persicae, Macrosiphum euphorbiae, Aulacorthum solani.*
Potato Virus X	Mechanical transmission, hands, clothing equipment etc. , Contact of plants in the field. Chewing insects e.g. grasshoppers. Zoospores of *Synchytrium endobioticum* (wart pathogen). Beetles
Potato Virus A	Aphid transmission e.g. *Myzus persicae, Acrthosiphon pisum, Rholpalosiphum padi* etc. Mechanical transmission
Potato Virus V	Aphid transmission e.g. *Myzus persicae, Acrthosiphon pisum, Rholpalosiphum padi, Brachycaudus helichrysi, Macrosiphum euphorbiae, Rhopalosiphoninus latysiphon*
Potato Mop Top Virus	*Spongospora subterranea f.sp. subterranea* (Sss), a soil borne pathogen causing powdery scab disease. Mechanical transmission, grafting.
Tobacco Rattle Virus	Nematode transmission e.g. *Paratrichodorus anemones, P. hispanus, P. pachydermus, P. allius, P. teres, P. tunisiensis, Trichodorus primitivus, T. similis, T. viruliferous, T. cylindricus, Nanidorus nanus, N. minor.*
*Potato Yellow Blotch Virus**	Mechanical transmission. Likely to be transmitted by aphid species

*As a consequence of the recent finding of PYBV there is currently no evidence in the literature concerning transmission, but it is likely to be transmitted by aphids due to its similarity to PVA.

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Potato Leaf Roll Virus (PLRV)

Potato Leaf Roll Virus is transmitted predominantly from plant to plant by peach-potato aphids (*Myzus persicae)* (Beemster, 1987) in a persistent manner, and resides in the phloem sap of plants. The virus is acquired from infected plants by aphids feeding on the plants and ingesting sap. The ability of an aphid to transmit leafroll virus is then delayed for several hours because the virus has to pass through the digestive system of the aphid and enter its saliva. Consequently, only aphids which colonise potato will be able to effectively transmit PLRV. PLRV can be artificially transmitted by stem grafting, but transmission via tuber grafting is difficult. PLRV is the only potato virus that is transmitted in a persistent manner by *M. persicae* with the efficiency of 10-80%. Much of the spread of PLRV occurs in early spread (May to early July) and thus an early spread of *M. persicae* means a greater incidence of PLRV (Garg & Khurana 2003). Transmission of PLRV is temperature dependent, with the greatest levels of infection occurring when plants were maintained at 25°C (Chung *et al.,* 2016).

Potato Virus Y (PVY)

In the field, PVY is transmitted by vegetative propagation of infected daughter tubers from an infected plant or by aphids that transfer the virus from an infected plant to an uninfected plant. Over 40 species of aphid are known to be able to transmit PVY (Edwardson and Christie, 1997; Ragsdale *et al*., 2001; Quenouille *et al*., 2013), although transmission efficiencies do differ depending on the aphid species. PVY is a non-persistent/non-circulative virus and thus rapidly infects the plant (in less than one minute) via an aphid vector by attaching to the stylet of the aphid (Pirone and Perry, 2002; Radcliffe & Lagnaoui, 2007). Non-persistent viruses can be passed onto another plant within a few minutes during aphid feeding. As a consequence, winged aphids which briefly probe a plant to determine whether they are suitable hosts, have the potential to spread the viruses quickly. These winged aphids may be non-potato colonising or potato colonising aphids and it is now widely considered that non-colonising aphids have a major role to play in the spread of PYV (Pickup *et al.,* 2009). In 2016, it was discovered that PVY^{O} infection of *N. benthamiana* could be enhanced by growing the plants at 20°C and that temperatures of 25°C and above significantly reduced PVY^{O} accumulation in the plant over time. This finding was found to also correlate with an increased level of RNA silencing, suggesting that RNA-silencing mediated plant defences are more active at higher temperatures. In addition, a recent report (Del Toro *et al.,* 2015) showed that the lower PVY and Potato Virus X titres in leaf disks at higher temperatures were not attributable to inactivation of viral suppressors but by increased antiviral silencing at higher temperatures.

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Potato Virus A (PVA)

As in the case of PVY, *Potato Virus A* (PVA) is transmitted by aphids in a non-persistent manner. At least ten aphid species are vectors, but the more important are *Aphis frangulae, Aulocorthum solani, Macrosiphum euphorbiae* and *Myzus persicae*. PVA is not seed-transmitted, but can be transferred mechanically (Ahmadvand, 2012). A recent study to determine the effects of temperature on acquisition of *Potato Virus A*, found that the optimum temperature for proliferation of PVA in *Nicotiana benthamiana*, is 20°C during early infection (Chung *et al.,* 2016). Chung *et al*., also found that symptom attenuation and reduced PVA accumulation occurred over time when plants were grown at temperatures over 25°C and that this phenomenon correlated with significantly increased RNA silencing.

Potato Virus V (PVV)

PVV is a potyvirus and is transmitted in a non-persistent manner by aphids. Its most important vector is *Myzus persicae* and can be transmitted by the aphid after acquisition access periods of 15-30 seconds and inoculation access times of 15 minutes (Fribourg & Nakashima, 1984). It can also be transmitted by the aphids *Brachycaudus helichrysi, Macrosiphum euphorbiae* and *Rhopalosiphoninus latysiphon* (Calvert *et al*., 1980; Bell, 1982; Bell, 1983).

Potato Yellow Blotch Virus (PYBV)

Potato yellow blotch virus (PYBV) is a new species of the genus *potyvirus*, first characterised in 2018 (Nisbet, *et al.,* 2019). Transmission tests are still to be carried out on this newly identified virus to determine the full range of vectors and plant hosts, but as a *potyvirus* extremely similar to PVA, it is likely to be transmitted by aphids in a non-persistent manner and maintained by vegetative/tuber propagation in potato.

Potato Virus X (PVX)

Potato Virus X (PVX) is mechanically transmitted by a number of methods, such as sap inoculation, stem and tuber grafting, grasshoppers and other chewing insects (Beemster & Rozendaal, 1972). PVX was also proven to be transmitted by zoospores of *Synchytrium endobioticum* (Munro, 1981). Damadi *et al*., (2005) demonstrated that infectivity of PVX could be reduced if infected sap was diluted to 10^{-6}, 10 minutes at 70°C and 10 weeks at room temperature.

Potato Mop Top Virus (PMTV)

Spongospora subterranea f.sp. *subterranea* (*Sss*) is a soil borne pathogen primarily causing powdery scab disease in potato. It is also the vector for transmission of *Potato Mop Top Virus* in potato cultivars (Arif *et al.,* 1995). PMTV can persist for an extended period of time in the

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resting spores of *Sss* and can remain infective for 18 years without the presence of a potato crop, which can make management of the disease challenging (Calvert, 1968; Kirk, 2008). The occurrence of *Sss* has been reported from all potato growing regions of the world. Transmission of PMTV is dependent on temperature and precipitation. The optimal temperature range for PMTV transmission is 12-20°C and little or no transmission will take place at temperatures over 24°C (Carnegie, *et al*., 2010). In addition, if precipitation is between 760mm or higher then infection will also be increased (Cooper and Harrison, 1973). The lifecycle of *Sss* has two stages; the first stage is the formation of resting spores called cystosori. During the second stage the cystosori will germinate and release zoospores that infect the roots, stolons and tubers. During infection, zoospores also transmit PMTV into host plants. PMTV can also be transmitted to some hosts by grafting or mechanical inoculations. PMTV cannot be transmitted via aphids.

Tobacco Rattle Virus (TRV)

Tobacco rattle virus (TRV) is a member of the genus *Tobravirus*, a group of plant viruses that are transmitted by Trichodorid nematodes (*Trichodorus* spp. *Paratrichodorus* spp. *Monotrichodorus* and *Allotrichodorus*) (Robinson and Harrison, 1989). Trichodorid nematodes are root migratory ectoparasites, and are able to pierce plant cells using a long arcade stylet. Trichodorid nematodes thrive best in sandy soil (sand fraction between 80-90% and a silt fraction < 10%) and are sensitive to low soil moisture (Mojtahedi and Santo, 1999). Generally under field conditions Trichodorids reproduce from spring to autumn and can complete their lifecycle in 22 days at 22°C (Block (2015) AHDB potatoes Report R433). Nematode vectors become viruliferous after feeding for 15-60 minutes. TRV virus particles are retained on the lining of the nematode pharynx and the virus is lost at each moult. Free living nematodes will usually moult 4 times during their lifecycle and the virus does not pass through the egg stage. They are able to move in the soil and are thought to escape chemical control measures, by migrating from depths where fumigants have reduced effect to cause damage on the plant (Weingartner *et al*., 1983). Crop rotations are thought to have little effect in controlling the vector. Nematode species which are considered to be most relevant to UK agriculture are *T. primitivus, T. similis, T. viruliferous, P. anemoes, P. pachydermus, P. teres* and *N. nanus.*

Alternative/diagnostic host species

Potato Virus Y (PVY)

Potato Virus Y has a wide host range, with the capability of naturally infecting more than 60 plants in more than nine families, including 14 genera of Solanaceae, such as pepper, tomato, tobacco and eggplant (Kerlan, 2006). PVY is also able to infect members of the

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Chenopodiaceae and Leguminosae families (Thornberry, 1966). Diagnostic species include *Capsicum frutescens* cv Tabasco (pepper), where PVY causes mild to severe mottle but no wilting (unlike tobacco etch virus), *Datura stramonium*, which is immune to all strains of PVY (unlike tobacco etch virus), *Nicotiana glutinosa,* where PVY causes mild to severe mottling and *N. tabacum*, where all PVY strains cause vein clearing followed by mottling, except for the necrotic strain. Combined infection with PVX and PVY produces a synergistic 'spot necrosis' reaction which has been used to detect the presence of PVY. In addition, *Solanum tubersoum* cv Saco is useful for separating *Potato Virus Y* from *Potato Virus X*, as this cultivar is almost immune (Benson & Hooker, 1960). *N. glutinosa* is used to maintain the virus and *N.tabacum* cv Wisconsin Havana 425 is used to purify PVY.

Potato Leaf Roll Virus (PLRV)

Potato Leaf Roll Virus is generally hosted naturally by Solanaceae species, but some non-solanaceous plants such as *Amaranthus caudatus*, *Celosia argentea*, *Gomphrena globosa* and *Nolana lanceolate* are susceptible (Natti *et al.,* 1953). *Physalis floridana* and *Datura stramonium* are useful for diagnostics, propagation and purification (Kojima *et al.,* 1969; Peters & Van Loon, 1968). They exhibit interveinal chlorosis and variable amounts of stunting, depending on the strain and temperature. *Physalis floridana* is also used to test the transmitting ability of aphids that have acquired the virus (Duffus, 1964; Mackinnon, 1965).

Potato Virus A (PVA)

Natural hosts are limited to the Solanaceae family. A range of diagnostic species are available, such as *Nicotiana tabacum* cv Samsun (vein-clearing and diffuse mottle depending on strain) and cv. 'White Burley' (vein-clearing and dark green vein-banding depending on strain), *Nicandra physalodes* (Slight vein-clearing and mottle to severe necrosis, rugosity and stunting, depending on the virus strain), *Lycopersicon pimpinellifolium* (Systemic necrosis and plant death) (MacLachlan *et al.,* 1953). *N. tabacum* cv Samsun is a good source for purification (Bartels, 1954) and *Nicandra physolodes* is used for aphid transmission tests, due to its high susceptibility to infection by *Myzus persicae*.

Potato Virus V (PVV)

Natural infection has only been detected in potato. A series of studies in the 1980's carried out sap inoculation of 31 different plants species in ten other plant families, but no infection was detected (Calvert *et al.,* 1980; Fribourg & Nakashima, 1984; Jones & Fuller, 1984). Diagnostic species include *Nicotiana clevelandii, N. glutinosa, N. occidentalis* (systemic vein clearing, vein banding and mosaic), *N. debneyi* (diffuse chlorotic spots in inoculated leaves, systemic vein clearing, vein banding, mosaic, and chlorotic spots and rings) and *N. tabacum*

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cv White Burley (faint systemic vein clearing, chlorotic spotting, mosaic and vein banding). Potato cultivars Estima and Desiree are also used for diagnostic purposes, as they develop faint mosaic and mottle after PVV inoculation respectively. In addition, Maris Piper and Pentland Crown develop necrotic spots in inoculated leaves and severe systemic necrosis. *N. occidentalis* seedlings are useful for aphid transmission tests (Fribourg & Nakashima, 1984).

Potato Virus X (PVX)

Potato Virus X is able to infect more than 240 species in 16 different families, however the majority of hosts are in the Solanaceae family (Purcifull & Edwardson, 1981). Diagnostic and propagation species available are *Nicotiana tabacum* (necrotic ringspots in first infected leaves or chlorotic/necrotic mottling, mosaic or veinal chlorosis in later infected leaves) and *Datura stramonium* (small chlorotic rings followed by mottling, veinal chlorosis/necrosis).

Potato Mop Top Virus (PMTV)

In addition to potato, PMTV (Jones and Harrison, 1972) can infect weeds as well as crop species belonging to families such as *Solanaceae, Chenopodiaceae* and *Tetragoniaceae* (Jones and Harrison, 1972; Andersen *et al.,* 2002). A large number of other host plants such as *Chenopodium amaranticolor, Nicotiana debneyi* and *N. tabacum* have been identified by artificial inoculation (Harrison and Reavy, 1974). Tomato (*Lycopersicon esculentum* Mill.) is also known to obtain PMTV through vector transmission. The common weed, black nightshade (*Solanum nigrum* L.), is a major reservoir for PMTV during the years when the potato is not cultivated (Andersen *et al.,* 2002).

Tobacco Rattle Virus (TRV)

Tobacco Rattle Virus has the widest known host range of any plant virus, with more than 400 species in more than 50 dicotyledonous and monocotyledonous families which can be infected experimentally, although many infections do not become systemic (Uschdraweit & Valentin, 1956; Noordam, 1956; Schmelzer, 1957). Alternative hosts include ornamental plants (gladiolus, narcissus, tulip, aster and other species), sugar beet, tobacco and arable weed species (field pansy, knotgrass, groundsel, shepherd's purse and chickweed). Diagnostic species include *Chenopodium amaranticolor* (necrotic local lesions, some tending to spread, develop in 3-5 days; not systemic), *Cucumis sativus* (chlorotic/necrotic local lesions; not systemic), *Nicotiana tabacum* cv. Samsun NN (necrotic spots or rings in inoculated leaves, and sporadic systemic distortion and/or necrosis. Symptoms vary depending on environment), *Phaseolus vulgaris* (pin-point, necrotic local lesions; not systemic) and *Pisum sativum* and *Vicia faba* (small necrotic local lesions; not systemic).

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Infection of *Nicotiana clevelandii* with TRV is systemic and thus is a good host for maintaining cultures and as a source of virus for purification. *Petunia hybrid, N. tabacum* 'White Burley' and *Cucumis sativus* are useful hosts for vector transmission tests.

Table 3. Alternate host species for key potato viruses in the UK. The most common alternate or diagnostic host species for the key viruses only. This list should not be considered exhaustive.

Virus	Alternate/diagnostic host
Potato Virus Y	*Nicotiana spp., Capsicum spp., Lycopersicon esculentum, Physalis floridana, Solanum spp., Erodium cicutarium, Geranium pusillum, Lactuca purpureum, Lamium serriola, Chenopodium spp.*
Potato Leaf Roll Virus	*Nicotiana spp., Solanum spp. Nicandra physalodes, Physalis floridana* (Rydb*.), P. angulate, Datura stramonium* L. *D. tatula,* and *Lycopersicon esculentum, Ullucus tuberosus Calda. Amaranthus spp., Celosia argentea, Gomphrena globose and Nolana lanceolata.*
Potato Virus X	*Lycopersicon esculentum, Nicotiana spp., Datura stramonium, Solanum spp., Hyoscyamus niger, Cyphomandra betacea, Gomphrena globose, Petunia sp., Crimson clover (Trifolium incarnatum).*
Potato Virus A	*Nicotiana tabacum, Nicanda physolades, Lycopersicon pimpinellifolium, Solanum demissum x S. tubersoum.*
Potato Virus V	*Nicotiana spp. Solanum tuberosum.*
Potato Mop Top Virus	*Nicotiana spp., Chenopodium album, Solanum nigrum*
Tobacco Rattle Virus	*Chenopodium amaranticolor, Cucumis sativus, Nicotiana spp., Phaseolus vulgaris, Pisum sativum, Vicia faba, Petunia hybrida, Beta vulgaris*.

Detection and Identification of Viruses

Detection and Identification Methods Based on Biological Properties

Symptomology

Numerous herbaceous plants are reported as susceptible indicator host plants, producing characteristic symptoms to a range of potato viruses in response to systemic infection or local lesions when transmitted mechanically. For example, species such as *Chenopodium amaranticolor, C. annuum, Physalis floridana, C. album, Nicotiana glutinosa, N. rustica, N. tabacum cv.* Samsun*, N. benthamiana, Datura stramonium, D. metel, D. stramonium, Solanum demissum x, S. tuberosum Gomphrena globosa* and *Lycopersicon esculentum* were all used as test plants for potato viruses by Abbas *et al.,* 2012. Further details on virus specific diagnostic species can be found in Table 3.

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PVY is a single stranded positive sense RNA virus of 9.7kb length and is prone to high mutation rates and recombination (Tromas and Elena, 2010). As a consequence, the complex of PVY strains can be distinguished on the basis of their biology (i.e. symptoms they elicit on indicator plants), serology and genome sequence (for a review please see Singh *et al*., 2008; Karasev and Gray, 2013).Traditionally PVY is diagnosed via examination of the symptoms elicited on plants (Singh *et al.*, 2008). These symptoms include veinal necrosis in tobacco, potato tuber necrotic ringspot disease on potato tubers and/or their ability to trigger a hypersensitive response in potato cultivars carrying various resistance genes ($_{Nc,\ Nytbr,\ Nz}$). Symptoms caused by PVY infection on potato depend on the virus isolate, host cultivar, environmental conditions and whether they are produced by aphid-mediated horizontal transmission or vertical transmission through infected tubers (Draper *et al.*, 2002).

Detection and Identification Methods Based on Viral Coat Protein

Serological detection of potato viruses relies on detection of coat protein (virus particles) with polyclonal (PAb) or monoclonal antibodies (MAb) and is commonly carried out using the enzyme-linked immunosorbent assay (ELISA) (Clark and Adams, 1977; Keller *et al.,* 2005). Routine screening for viruses such as, PVY, PLRV, PVX, PVA, PVS, PVM PVV, TRV and PMTV is an essential part of the Seed Potato Classification Scheme (SPCS) in Scotland, carried out by SASA (Science and Advice for Scottish Agriculture), and serological assays are often used to detect prevalence of common viruses due to its relative sensitivity and its suitability to high throughput automation (Pickup *et al.,* 2009). Due to the increasing number of PVY variants, it has been essential to develop serological assays which can differentiate between the strains. Commercial monoclonal antibodies, MAb1128, MAb1129, and MAb1130, recognize the viral coat protein (CP) of PVY and distinguish PVY^N strains from PVY^O and PVY^C strains, or detect all PVY strains, respectively. In 2014, a detailed Mab epitope analysis was carried out for MAb1128, MAb1129, and MAb1130 which pinpointed the critical residues required for PVY recognition. This detailed serological analysis allows sequence-based identification of PVY serotypes, and can help predict whether particular isolates could escape detection or should be detected using anti-CP Mab and other novel detection tools (Tian *et al.,* 2014).

Precipitation and agglutination tests

Historically, visual inspection of disease symptoms was the first major step in virus detection. However, diagnosing viral diseases by symptoms alone can be challenging, as some viruses can be asymptomatic in some plant material and similar visual symptoms can be produced by nutrient deficiencies or other abiotic or biotic factors. These diagnostic challenges can be overcome with the use of indicator hosts, however, virus detection using indicator hosts

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requires significant time, labour and space. Therefore, alternative techniques based on serology and histochemical tests were standardized for detection of viruses during the 1960s and1970s. The most commonly used serological tests at this time were the precipitin and chloroplast agglutination. These tests were used effectively in detecting PVX, PVS and PVM, however the technique requires a large quantity of antisera and can only detect viruses with high viral titre (Hampton *et al.,* 1990). The limitations of chloroplast agglutination to detect potato viruses were overcome by the development of ELISA assays in the 1980s which are used widely.

Microscopy-immunoelectron and electron

Electron microscopy (EM) is used for detection of the size and shape of a particular virus from infected plant tissue. *Potato Virus Y* was characterised in the 1950s via transmission electron microscopy of purified and unpurified sap preparations from infected potato leaves, using a negative staining method (Bawden and Nixon, 1951). In 2011, Kogovšek *et al.* used electron microscopy as part of an integrated approach, including real-time PCR and In Situ hybridisation, to localise PVY^{NTN} virus particles, viral RNA and cytoplasmic inclusion bodies. This study revealed an uneven distribution of the virus throughout the plant after early systemic infection, indicating tissue- or organ-specific mechanisms employed by either the virus or by the plant to determine the level of virus accumulation. Immunosorbant Electron Microscopy (ISEM) is a highly sensitive technique which was developed by combining electron microscopy and serology in order to detect plant viruses (Gars and Khurana, 1991) and is x1000 more sensitive than conventional electron microscopy. This technique is particularly useful for detection of phloem-restricted viruses such as PLRV, which are difficult to detect via electron microscopy or ELISA due to their low titre.

Immunoblotting

In immunoblots or dot-blot assays, antibodies or virus particles bound to nitrocellulose membrane filters are used. Dot blot ELISA tends to be rapid, easy to perform and are often more sensitive than ELISAs carried out in microtitre plates. Immunoblot assays use the same reagents used in microtitre plate ELISAs, except that the substrate produces an insoluble product which precipitates onto the membrane. Positive reactions can be determined visually. Assays in which antibodies or antigens are bound to nitrocellulose or nylon membranes have been used to detect PVS, PVX, PVY and PLRV (Smith and Banttari, 1987). This method has proven effective in confirming the presence of PVX and PVY from tubers in the field (Bravo-Almonacid *et al*., 1992).

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Detection of plant viruses using biosensors

Over the last ten years a number of articles have been published demonstrating the effectiveness of antibody-based biosensors, due to their high sensitivity, selectivity and rapid response times. The biosensors enable pathogen detection in air, water and seeds with different platforms for greenhouses, in-field and postharvest storage of processors and distributors of crops and fruits (Skottrup *et al.,* 2008). Most antibody-based biosensors use one of the following types of electrochemical transducers: amperometric, potentiometric, impedimetric and conductometric. Non-electrochemical transducers include surface plasmon resonance (SPR), quartz crystal microbalance (QCM) and cantilever-based sensors (Zeng *et al.,* 2013; Eun *et al.,* 2002). Perdikaris *et al.,* (2011) published a novel portable biosensor system for the detection of plant viruses, based on immobilized 'Ver' cells which carry virus specific antibodies on their membranes,. This technique was designated the 'High Throughput Bioelectric Recognition Assay (BERA-HTP). The sensor is based on live, mammalian cells, the membrane of which has been artificially saturated with antibodies specific to plant viruses. The attachment of the specific virus to the antibodies caused a virus-specific change of the cell membrane electric potential. The BERA-HTP method was able to detect purified *Potato Virus Y*, *Cucumber mosaic virus* and *Tobacco Rattle Virus*, in single, as well as mixed, infections in different host species. This study demonstrated an important step towards developing a portable plant virus detection system suitable for in-field application.

Detection and Identification Methods Based on Viral Nucleic Acid

Nucleic acid hybridisation assays

Molecular hybridisation can be used to detect plant viruses and is based on the interaction between purines (A=T) and pyrimidine (G≡C) and results in the formation of a stable hybrid between the target sequence of the virus and the molecular probe. The dot-blot hybridisation is the most common technique which utilises the interaction between the target nucleic acid and virus specific probes (Pallás *et al.,* 1998). Nonradioactive riboprobes were used for successful detection of PVS, PVX (Eweida *et al.,* 1999), PVY and PLRV (Hopp *et al.,* 1998).

In situ hybridisation is a type of hybridisation that uses a complementary molecular probe to detect a specific DNA/RNA sequence in a section of tissue, in order to detect and localise the target sequence or pathogen. In 2011, Kogovšek *et al.,* developed an in situ hybridisation method to detect the distribution of PV-Y^{NTN} in systemically infected potato plants of the highly susceptible cultivar Igor. They analysed different plant organs and tissues for virus presence and accumulation at the cellular level. PVY was shown to accumulate in all studied leaf and stem tissues, in shoot tips, roots and tubers; however, the level of virus accumulation differed

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depending on the organ or tissue type. The highest amounts of viral RNA and viral particles were found in symptomatic leaves and stems.

Polymerase chain reaction technology

Rapid developments in PCR-based technologies and sequencing of isolates since the late 1980s have made it possible to rapidly characterise and classify potato virus strains based on molecular characteristics. RT-PCR assays have been developed to detect strains of PVY, PLRV, PVX, PVA, PVS and PVM (Nie *et al.,* 2008; Abbas *et al.,* 2012Thole *et al.,* 1993, Singh. 1999, Nie & Singh 2002, Rupar *et al.,* 2013). PVY is a rapidly evolving virus, where both variation within strains and recombination events between strains occurs. This phenomena has led to numerous PCR based detection assays being developed to identify different mixtures of common and recombinant PVY strains in multiplex assays (Nie & Singh, 2002; Nigh & Singh, 2003; Boonham *et al.,* 2002; Lorenzen *et al.,* 2006; Crosslin and Hamlin, 2011). Analysis of the genome using molecular biology methods has brought a new understanding of PVY at a nucleotide level. It is now known that at the nucleotide level, multiple isolates of the PVY^{O} and PVY^{N} groups differ from each other by approximately 8% along their genomes (Karasev *et al.,* 2010). Molecular genome characterization has also identified where recombination events on PVY genomes have occurred to create new strains such as $PVY^{N\text{-}Wilga}$ and PVY^{NTN} (Boonham *et al.,* 2002) and has revealed that in many countries the majority of isolates are now recombinants between PVY^{O} and PVY^{N} (Yin *et al.,* 2012. Blanchard *et al.* 2008).

Microarrays or DNA chips were first designed to study gene expression or identify single-nucleotide polymorphisms (SNP) and have now become a standard tool for diagnostics of human and plant pathogens. Microarrays hybridise fluorescently labelled sequences (targets) to their complementary sequences spotted on a solid surface, acting as probes. The key advantage of the technique is the ability to detect many pathogens simultaneously, with the chip being made up of tens of thousands of DNA probes. Several studies have been published where microarray-based detection has been used to diagnose plant viruses (Pasquini *et al.,* 2008; Nicolaisen, 2011). In potato, Boonham *et al.,* (2003) published a study whereby PVY, PVA, PVX and PVS were detected in single and multiple infections using a microchip consisting of spotted arrays of PCR products. However, developing microarray chips which are comprised of PCR products is extremely laborious and expensive. Therefore, in 2005, Bystricka *et al.,* went on to describe a microchip using short synthetic single-stranded oligomers (40 nt) instead of PCR products as capture probes for detection of PVA, PVS, PVM, PVX, PVY and PLRV, in both single and mixed infections. Sip *et al.,* 2010 also reported

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oligonucleotide microarray for the detection of mixed infections of PVA, PVS, PVM, PVX, PVY^{O}, PVY^{N}, PVY^{NTN} and PLRV.

Immunocapture PCR is a combination of serological and molecular tools in a single detection technique, in which the virus particles are captured (via the protective protein coat) and amplified using PCR (Nolasco *et al.,* 1993). This method is x250 more sensitive than traditional PCR and removes the requirement to purify the virus to eliminate plant cell components which interfere with the PCR reaction. This technique has been successfully developed to detect PLRV (Leone *et al.,* 1997), however not suitable for high throughput diagnosis.

Novel technologies

Hyperspectral sensors and imaging techniques

Hyperspectral sensors and imaging techniques have shown a great deal of potential for detection, identification and quantification of plant disease in the field, and understanding of plant-pathogen interactions (Mahlein *et al.,* 2018). Several studies have shown that hyperspectral analysis is extremely valuable in a wide range of crops and diseases, ranging from detection at the tissue level through to whole crop canopy (Sankaran *et al.,* 2010; Mahlein *et al.,* 2012; Wahabzada *et al.,* 2015; Thomas *et al.,* 2018). Much of the work to date has been carried out on fungal diseases, however in recent years attention has turned to developing similar methodology to detect viral diseases in crops. For example, in 2018, Griffel *et al.* reported the successful detection (89.8% accuracy) of PVY in potato plants using spectral signatures that were acquired with a hand-held device. Most recently, Polder *et al.,* published a new method in March 2019, which uses deep learning on hyperspectral images to detect *Potato Virus Y* in seed potatoes. They have used a novel fully convolutional neural network (FCN) to successfully detect PVY in potato based on hyperspectral image data. This method was found to have an accuracy of 75-92%, slightly lower than the accuracy of a crop expert (93%) and has the potential to be used to scan an entire field. Moslemkhani *et al.* (2019) have also investigated spectral reflectance data obtained from PVY-infected and healthy potato plants (Agria and Milva cultivars) for the development of a spectral detector for a rapid and non-destructive PVY detection system. A review of hyperspectral image analysis techniques for the detection and classification of the early onset of plant disease and stress is given by Lowe *et al.* (2017).

Modelling and decision support systems

Decision support systems for potato farmers in the UK are centred around aphid monitoring networks provided by AHDB, FERA, SASA and Rothamsted Research and aphid predictions based on average winter temperatures. Zhou *et al.,* (1995) determined that winter

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temperature is the dominant factor affecting aphid migration phenologies for aphid species *Brachycaudus helichrysi, Elatobium abietinum, Metopolophium dirhodum, Myzus persicae* and *Sitobion avenae,* with a 1°C increase in average winter temperature advancing aphid migrations by 4-19 days depending on the species. This research became central for aphid migration predictions in the UK via SASA, AHDB and Rothamsted Research and plays a major part in informing UK potato farmers of the aphid and aphid-borne virus risk. Abundance of *Myzus persicae* in the preceding year has also been found to correlate to high incidence of *Potato Leaf Roll Virus* in the following year (PickUp *et al.,* 2009).

Outside of the UK, Steinger *et al.,* (2015) utilised virus incidence data from seed potatoes (1989-2012) collected within the Swiss seed certification programme to develop a decision support system capable of forecasting virus spread in seed potatoes, using flight activity data of aphid vectors. Model selection techniques were used to regress year-to-year variation in the incidence of potato viruses (largely *Potato Virus Y* (PVY)) against the abundance of winged aphids obtained in suction traps, to identify the most important vector species. Surprisingly, the abundance of *Myzus persicae*, often considered the main vector of PVY, was not correlated with virus incidence in this study, suggesting that the early migrating aphid *B. helichrysi*, is the main vector of PVY in Switzerland. Winter temperature (January–February) was also found to be positively correlated to the abundance of *B. helichrysi* in early summer, as well as with post-harvest virus incidence.

San Choi *et al.,* (2017) studied the effect of temperature on the rate of systemic infection of potatoes by PVY. Systemic infection was observed only between 16°C and 32°C and the period of systemic infection decreased from 14 days at 20°C to 5.7 days at 28°C. A systemic infection model was successfully constructed based on experimental data, with the potential to predict the progress of systemic infections by PVY in potato plants and to construct future epidemic models.

Chemical and cultural management options

Insecticides are efficient in controlling aphid populations, which are the vectors of diseases such as PVY, however they rarely limit virus spread in the field due to the short time needed to transmit non-persistent viruses (Shanks & Chapman, 1965; Gibson *et al.,* 1982; Boiteau *et al.,* 1985; Boquel *et al.,* 2014). It is often the case that aphids have transmitted the virus before the insecticide has had time to take effect (Perring *et al.,* 1999). It is therefore important to develop an integrated management approach which combines traditional control methods such as aphid monitoring and pesticide application with alternative cultural control methods. There are a number of possible cultural control methods which are used to minimise aphid populations in the UK and prevent the spread of key viruses, such as *Potato Virus Y*. We will

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consider the literature surrounding the following methods currently being implemented within the UK seed and ware potato industry:

i. Aphid monitoring and chemical control
ii. Straw mulching
iii. Crop borders
iv. Mineral oil spraying

i. Aphid monitoring and chemical control

Aphid monitoring

Monitoring of aphid populations throughout the UK and in the potato crop is carried out via the AHDB yellow water-pan trap (YWT) network operated by Fera and the Rothamsted/SASA suction-trap (ST) network. The Rothamsted Insect Survey (RIS) is a national capability funded by BBSRC that has been collecting data on the migration of moths and aphids in the UK since 1964. The data from the suction trap network is available at https://insectsurvey.com and is summarised via the weekly AHDB Aphid news bulletin. The YWT data is summarised at http://aphmon.fera.defra.gov.uk/.

Seed potatoes produced and marketed in GB must be classified under the Seed Potato Classification Scheme (SPCS). SASA (Science and Advice for Scottish Agriculture) is the certifying authority for seed potatoes in Scotland and administers the scheme. APHA implements the scheme in England and Wales. Crop inspections for symptoms of pests and diseases, including potato viruses, are carried out during the season. In Scotland samples are collected for diagnostic testing for key viruses. Information on the abundance of aphids is also collected to help monitor the risk of virus spread during the potato growing season. Three Scottish suction traps (12.2 m above the ground) are in operation from March to December, to provide a standardized measure of regional aphid activity. The traps are located at Gogarbank and Dundee on the east coast and Ayr on the west coast and data is updated weekly, providing information on key species and regional risks of PVY transmission (Pickup *et al.,* 2009; http://www.sasa.gov.uk/wildlife-environment/aphid-monitoring/aphid-bulletins). The data for Dundee and Gogarbank is summarised in the Aphid News.

Insecticide resistance and chemical control

The development of insecticide resistance by insect and mite pests is a constant threat to the sustainability and competitiveness of potato production around the world. Peach-potato aphid (*Myzus persicae*) and potato aphid (*Macrosiphum eurphorbiae*) are the main colonising aphid

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pests of potatoes, which transmit persistent viruses, such as *Potato Leaf Roll Virus* (PLRV). *Myzus persicae* is well known for its insecticide resistance, with the first report dating back to 1955 (Anthon, 1955), and the metabolic and target site resistance mechanisms have been researched and monitored for many years. Resistance is now reported to most classes of insecticide, including the organophosphates, carbamates, pyrethroids, cyclodienes and neonicotinoids (see Bass *et al.,* 2014 for review). Insecticide resistance monitoring in potato crops in 1996 found aphids with high esterase, MACE and kdr resistance mechanisms widely distributed on potato crops in eastern England. However, carboxylesterase and MACE resistance levels appeared to decline to low levels by 2000, with a new form of *M. persicae* arriving in the UK in 2001, carrying MACE resistance and a 'new' form of super-kdr conferring resistance to pyrethroids. Resistant aphids resurged in central and eastern Scotland and are now widespread throughout the UK. *M. persicae* expressing MACE resistance in the UK show high resistance to pirimicarb. Neonicotinoid Resistance (Nic-R++) is only found in the southern European countries to date and confers strong resistance specifically to neonicotinoids. Fortunately, to date no aphids in the UK have been identified with this form of resistance. Ongoing screening programmes in the UK have also shown no evidence of resistance to pymetrozine or flonicamid in *M. persicae* in the UK. (IRAG: https://ahdb.org.uk/irag).

There is currently no evidence of field resistance to insecticides in *M. euphorbiae*, however, increased levels of carboxylesterases have been detected in laboratory experiments of some individuals which have been collected from UK fields, suggesting that there is potential for resistance to develop. Insecticide resistance has also been identified in both *Aphis nasturtii* (buckthorn-potato aphid) and *A. gossypii* (melon aphid or cotton aphid), which are occasionally found on UK crops. Non-colonising aphid species which can transmit non-persistent, rapidly acquired potyviruses, such as PVY and PVA, are also at danger of developing insecticide resistance. Of the non-colonising aphid species affecting potato, the grain aphid (*S. avenae*) has been identified to carry target site resistance (kdr) to pyrethroids. Reduced sensitivity to pyrethroids also been demonstrated in the willow-carrot aphid (*Cavariella aegopodii*).

The key modes of action (MoA) available for use in the UK to control aphids are pyrethroids (3a), neonicotinoids (4a), tetronic and tetramic acid derivatives (23) and chordotonal organ modulators (29) (See Table 4). Almost all seed crops and many ware crops are treated for aphids. Pyrethroids, neonicotinoid and flonicamid insecticides can be used either alternately or in mixtures, depending on the products. It is not normally thought necessary in a standard season, to apply more than one insecticide application on ware crops, (including seasons where there is rapid build-up of aphids during the summer (usually late June) and natural

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enemies are also low), as aphid populations decline naturally mid-July. However, monitoring through the season is essential, as late aphid migrations can occur in some regions and seasons.

Table 4. Aphicides available for use on potatoes in the UK, along with mode of action (MoA), restrictions on use and notes on current UK resistance. Adapted from Insecticide resistance status in UK potato crops, IRAG (2018).

Mode of action (chemical group)	**Active ingredient(s)**	**Max permitted number of applications ***	**Peach-potato aphid resistance status in UK**
3a (pyrethroids)	Cypermethrin	2	Strong resistance widespread
3a (pyrethroids)	Esfenvalerate	4	Strong resistance widespread
3a (pyrethroids)	Lambda-cyhalothrin	No limit	Strong resistance widespread
4a (neonicotinoids) **	Acetamiprid	1 (ware), 2 (seed)	No resistance
4a (neonicotinoids) **	Thiacloprid	1 (ware), 2 (seed)	No resistance
23 (tetronic and tetramic acid derivatives)	Spirotetramat	4	No resistance
29 (chordotonal organ Modulators)	Flonicamid	2	No resistance

In 2018 and 2019 the UK potato industry saw the loss of two important active ingredients for controlling aphids: the neonicotinoid, thiamethoxam was withdrawn by the European Commission in April 2018, and the pyridine azomethine derivative, pymetrozine followed in April 2019. The loss of both active ingredients is likely to make management of insecticide resistance in seed and ware potatoes more challenging going forward.

ii. Straw mulching

In the literature it has been shown that the initial colonisation of aphids within a potato field is often concentrated at the field margins (Difonzo *et al.,* 1996; Carroll *et al.,* 2009). Döring & Chittka, (2007) hypothesised that the strong visual contrast between the crop canopy and bare ground at the margins acts to attract more aphids than homogenous cultivated land. Straw mulching, crop borders and intercropping make use of this phenomenon, by manipulating the contrast between the canopy and bare soil. Straw mulching has proven to be a powerful tool for minimising the spread of PVY and is known to reduce PVY incidence by 30% (Saucke & Döring, 2004; Kirchner *et al.,* 2014), with fewer winged aphids being captured in mulched plots in comparison to unmulched crops (Saucke & Döring, 2004).

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Aphids are thought to land on the straw or crop canopy indiscriminately due to the low contrast between the canopy and straw background, however when the aphids feed on straw they will leave the plot, and begin a 'rejection flight' (Kring, 1972; Döring *et al.,* 2004). This phenomenon is increased in early crop development (Saucke & Döring, 2014) and declines later in the growing season. However, large year-to-year variation has been observed in all multiyear trials with reductions in PVY incidence ranging from just 6% (Saucke & Doring, 2004) to up to 70% (Kirchner *et al.,* 2014).

iii. Crop Borders

With regards to the benefits of crop borders there are two possible mechanisms which can take place, the 'virus sink' effect (Boiteau *et al.,* 2009), where the aphid loses its ability to transmit the virus by the time it probes the border plants and the 'mechanical barrier' effect (Difonzo *et al.,* 1996; Fereres, 2000; Boiteau *et al.,* 2009), where the border forms a physical barrier between the aphid and the crop canopy. This technique is mainly used in the USA, where larger fields have more available space to implement the borders (Davis *et al.,* 2009). An alternative approach for countries which generally have smaller fields, as in Europe, is the use of intercropping, whereby the intercrop surrounds the potato plants and acts as mechanical barrier for aphids as well as a virus sink. Intercropping may also limit the spread of PVY by reducing the gaps in the canopy that are known to spread PVY (David *et al.,* 2009). Data from the first trial using oat intercropping was published in 2010 and demonstrated that the technique was effective at lowering PVY spread (Dupuis *et al.,* 2010).

iv. Mineral Oil Application

Spraying mineral oil onto the potato crop canopy is another PVY control option which is reported to lower the acquisition and retention of PVY by aphids (Wróbel, 2009; Boquel *et al.,* 2013). However, the efficacy of mineral oil in controlling the spread of PVY varies widely from 18% to 89% (with an average of 49%) depending on the study (Boiteau & Singh, 1982; Martin-Lopez *et al.,* 2006; Boiteau *et al.,* 2009; Hansen & Nielsen, 2012; Fageria *et al.,* 2014; Kirchner *et al.,* 2014; Mackenzie *et al.,* 2014; Steinger *et al.,* 2014). A comprehensive literature review of insecticide and mineral oil use in preventing the spread of non-persistent viruses in potato crops was written in 2010 by Al-Mrabeh *et al.* (supported by the AHDB). This review provided evidence for a reduction in potyvirus spread in the range of 30-60% when oil based treatments were compared to the untreated controls. The review concluded that the use of mineral oils in the UK crop was under-utilised and there was a lack of relevant research to support the use of this method in the UK as an alternative to insecticides. Subsequently, a four year project was funded to establish the effectiveness of mineral & vegetable oils in minimising the spread of non-persistent viruses in potato seed crops in Great Britain (Dawson *et al.* 2014). The overall aim of the project was to investigate the potential for mineral oils to

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be used as a control method for potyviruses (non-persistent viruses) in seed crops subject to the GB Certification system of growing crop inspection and control programmes for persistent viruses (*Potato Leaf Roll Virus*, PLRV) and potato late blight (*Phytophthora infestans*). It was concluded that oil based treatments did perform better than the control, with regards to PVA and PVY^N incidence, but significant year-year variation was observed. Importantly, it was concluded that applying mineral oil to potato crops did not interfere with statutory growing crop inspections and was a viable method for future control of aphids.

Breeding for aphid resistance and host resistance and/or tolerance

The modern potato (*S. tuberosum ssp. tuberosum*) originated from the introduction of cultivated Andigena (*S. tuberosum ssp. andigena*) to Europe from South America in 1570. The crop deteriorated over many years, through successive generations, and finally in 1921 Salaman (1921) determined that the decline was due to virus infection. This led to the start of breeding for virus resistance and the UK Statutory Seed Certification Scheme. Resistance breeding plays a major part in preventing yield losses cause by potato viruses. From the 1930's onwards considerable efforts were made to characterise virus resistance genes and phenotypes in *Solanum* species, and a comprehensive catalogue of known genes for resistance to potyviruses and PVX, their relationships and mapped positions is reviewed in Soloman-Blackburn & Barker (2001). There are several forms of virus resistance which can be incorporated into potato breeding programmes, such as resistance to infection, resistance to virus accumulation, resistance to virus movement. Some PVY/A resistances are temperature dependent, so climate change, location might affect variety resistance in potatoes. The key forms of resistance in potato breeding programmes; hypersensitive resistance (HR), extreme resistance (ER) and adult plant resistance will be reviewed below.

Hypersensitive resistance (HR)

Hypersensitive resistance is generally strain-specific and is a rapid defence response that results in death (necrosis) of the cells at the infection site, which prevents the infection from spreading further (Dixon *et al.,* 1994). Wild potato species are rich in genes for HR to potato viruses and many have been introgressed into cultivated potato and used in resistance breeding (Zimonoch-Guzowska *et al.,* 2013). However, due to the strain-specific nature of these resistance genes they can be overcome by new virus variants. It is therefore very important to phenotype the local PVY strains by inoculation of indicator potato cultivars containing specific HR genes, such as *Ny, Nc, Nz* (Jones 1990), so the most appropriate HR genes can be selected for cultivation. PVY strains controlled by HR genes *Ny, Nc* and *Nz* are categorised into strain groups PVY^o (ordinary strains), PVY^C (C strains), and PVY^Z (Z strains), respectively (Singh *et al.,* 2008). PVY strains that overcome all three genes are particularly

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problematic and are generally categorised as PVY^N, due to their ability to produce veinal necrosis in tobacco leaves (Singh *et al.,* 2008). Historically, PVY^N strains had mild impacts on potato crops and many cultivars were tolerant to them, but over the last 40 years more severe variants have become prevalent which cause severe mosaic and yellowing in leaves and necrosis of tubers. It is now known that these strains have evolved due to the recombination of PVY^N and PVY^O and are now classified as PVY^{NTN} or $PVY^{N\text{-}Wi}$. A large number of new recombinant strains have also been recently described, which cannot be controlled by *Ny, Nc* and *Nz* and do not induce veinal necrosis in tobacco. This strain group is now known as PVY^E (Singh *et al.,* 2008).

Extreme Resistance (ER)

Plants exhibiting ER to a potato virus show no or limited symptoms when inoculated with the virus, and only extremely low levels of virus can be detected, if any at all. ER is a dominant trait, with virus–specific R genes inhibiting virus multiplication, and provides protection against all strains of the virus and can, in some cases, show resistance to more than one virus. The genes designated *Ry* confer ER to PVY and are capable of controlling the widely spread, severe, recombinant PVY strains. Extreme resistance to PVY has been identified in *Solanum tuberosum L. group Andigena* (Ry_{adg}) (Muñoz *et al.,* 1975), *S. stoloniferum* (Ry_{sto}), *S. chacoense* (Ry_{chc}), *S. demissum* and *S. hougassi* (Cockerham, 1970) and several have been mapped to the potato genome via marker-assisted selection (Solomon-Blackburn & Barker, 2000). The *Rx* locus (multiple alleles) mapped to Ch XII by Bendahmane *et al.,* in 1997, confers extreme resistance to PVX in potato and has since been identified as a CC-B-LRR protein which targets the PVX coat protein (Bendahmane *et al.,* 2002).

Many attempts to introgress ER to protect against PVX and PVY have been made since the 1940s (Ross, 1954a, b, 1978, 1986; Davidson, 1980), but relatively few cultivars contain ER resistance to strains of either virus. There are several possible reasons for this. Firstly, ER genes have only recently been introduced into the *S. tubersoum* gene pool from a fairly limited number of sources, in comparison to HR genes which are more widely spread. In addition, *S. stoniferum*, the source of both Ry_{sto} and Ry_{sto}^{na} genes, does not cross freely. *S. tuberosum* and *S.stoloniferum* breeding lines, are generally male-sterile (Świeżyński, 1994; Ross, 1986) and highly susceptible to PLRV. It is also extremely time-consuming to select for ER when HR is present within the same population, as ER is epistatic to HR (Valkonen *et al.,* 1994). *Ry* resistant cultivars are available in Germany, Holland, Poland and Hungary, but as yet no UK-bred cultivars are known to have an *Ry* gene, which is likely to reflect local breeding

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priorities. Cultivars *with* Rx_{tbr}, Rx_{adg} or Rx_{acl} have been produced in several countries including USA, Germany, Argentina, UK and Ireland (Ross, 1986).

Breeding for virus resistance remains a high priority for potato breeders, as there is a continuing need to identify novel and durable virus resistance and develop cultivars with yields and quality acceptable to the processors and supermarkets. Major advances in genome sequencing and genotyping methods have resulted in the development of large genetic and phenotypic datasets that will enable more efficient and rapid breeding approaches. Hirsch *et al.,* (2014) developed Spud DB (http://potato.plantbiology.msu.edu/) for the scientific and breeding community to access the potato genome sequence and annotation datasets. The database also includes the SolCAP potato 8303 Infinium SNP array genotypic data and phenotypic data from a diversity panel of 250 potato clones, and thus represents a significant resource to aid the future advancement of potato breeding and research. Armstrong *et al.,* (2019) published a novel Diagnostic resistance gene enrichment sequencing (dRenSeq) method to identify the presence of functional nucleotide binding-leucine rich repeat (NLR) genes in tetraploid potatoes. This methodology has the ability to inform on (i) germplasm pedigrees (ii) complementary sources for NLR stacking (iii) the historic deployment of resistances (iv) the geographical differences in NLR deployments, and is the only available method to cost-effectively analyse multiple genotypes in crop breeding programs, identify germplasm with redundant NLRs, and to confirm transgene integrity in commercially available GM crops. This major advance has enabled rapid cloning of known NLR genes such as Ry_{sto} (Grech-Baran *et al.,* 2018, 2019) and help with the identification and characterisation of novel resistance genes, providing a means to improve the speed and efficiency of future disease resistance breeding in crops.

Mature Plant Resistance (MPR)

Mature Plant Resistance (MPR) to PVY in potatoes was first described by Debokx (1964) and is the phenomenum whereby plants can acquire some resistance to key viruses during aging. MPR is thought to be associated with a decrease in metabolic activity of the plant, which in turn reduces the translocation speed of viruses such as PVY and PLRV (Beemster, 1976, Debokx, 1964, Gibson, 1991, Barker, 1987, Whitworth et al. 2000). A significant decrease of ribosomes, glycoproteins and RNA content was observed in old leaves infected with PVY when compared to the youngest fully expanded leaves (Venekamp & Beemster, 1980, Venekamp et al., 1980). No evidence was found to associate dry matter, organic nitrogen, chlorophyll, soluble protein content and peroxidase activity with the occurance of MPR (Braber et al., 1982). Shibata et al., (2010) found evidence to suggest that the susceptibility of young tobacco plants when infected by *Phytophthora infestans* was due to a lack of salicylic

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acid (SA) signalling induction, suggesting that SA could play a significant role in signalling the status of MPR in the whole plant. This type of resistance is also strain –dependent, as it is more pronounced for control of PVY^O strains that PVY^N (Debokx, 1964). MPR varies depending on the variety of potato, for instance the cultivar Désiréé reaches MPR status significantly earlier than the Kind Edward, Record or Maris Piper (Gibson, 1991) and some varieties such as Russet Norkotah, do not appear to develop MPR at all (Zhang, 2014).

Genetic modification and CRISPR technology

Over recent years, virus resistance in potato has been engineered through a variety of different approaches ranging from simple plant breeding (reviewed in section above) through to advanced genetic engineering. RNA interference (RNAi)-mediated resistance which targets and silences the viral coat protein has been demonstrated in potato by several research groups, where single or multiple RNA viruses have been targeted with varying levels of success, such as PVY- resistance (Missiou *et al.,* 2004); PVY and PLRV-resistance (Chung *et al.,* 2013); and PVX, PVY and PLRV-resistance (Hameed *et al.,* 2017). Igbal *et al.,* (2016) identified 5 miRNAs which have the ability to target CI, Nia, Nib-Pro, HC-Pro, CP and VPg genes of PVY and could be used to develop PVY-resistant crops in the future. In addition, genetic engineering has also offered some promising solutions to controlling aphid vectors which transmit viruses like PVY, such as RNAi-or CRISPR-Cas9-mediated insect resistance (see reviews: Zhang J. *et al.,* 2017; Douglas, 2017).

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Virus Management in cereals

Current viruses present in the UK

A total of 13 viruses affecting wheat and nine viruses affecting barley have been recorded in the UK (Table 5). However, in a survey of UK wheat fields between 2009 and 2012 only *Barley yellow dwarf virus* (BYDV) and *Soil-borne cereal mosaic virus* (SBCMV) were found (Flint, 2015). The survey used real time reverse transcriptase polymerase chain reaction (qRT-PCR) to test for known viruses in 1356 samples. Most viruses were detected in southern England and Flint (2015) suggests that this was due to the greater area of wheat grown there than in northern England and warmer conditions encouraging the activity of vectors. The highest incidence of virus was in 2011/12, which had the warmest autumn, winter and spring. As described in the section Vectors of key viruses below, this would encourage the activity of important vectors. Several years with a warmer than average autumn, winter and spring have occurred since the survey (Met Office, 2019) and if this trend was to continue due to climate change than it is likely that plant viruses will become more problematic. Warmer conditions may also increase the impact of viral infection, within-plant virus spread and replication (Flint, 2014).

This review of cereal viruses will focus on the viruses already in present in the UK that are considered to be of (potential) economic importance. These are BaMMV, UK subgroups of BYDV, BaYMV, SBCMV and WDV.

Table 5. Summary of viruses reported from wheat and barley in the United Kingdom, together with, where available, information on the susceptible crop host ("Crop"), their mode of transmission and their vector. W = wheat, B = barley. Viruses highlighted in bold italics are further discussed in the present section.

Virus	Acronym	Crop	Mode of transmission	Genus
Agropyron mosaic virus	AgMV	W & B	Mites (*Abacarus hystrix*)	Rymovirus
Aubian wheat mosaic/"Bedford virus"	AWMV	W	Seed transmitted and possibly the soil-borne vector, *Polymyxa graminis*	Furo-like virus
Barley mild mosaic virus	BaMMV	B	Soil-borne vector, *P. graminis*	Bymovirus
Barley yellow dwarf virus	BYDV	W & B	Aphids (primarily *Rhopalosiphum padi* and *Sitobion avenae*)	Luteovirus
Barley yellow mosaic virus	BaYMV	B	Soil-borne vector, *P. graminis*	Bymovirus

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Cocksfoot mild mosaic virus	CfMMV	W & B	Aphids (e.g. *Myzus persicae*) and beetles (inefficiently)[2]	Sobemovirus
Cocksfoot mottle virus	CfMV	W & B	Cereal leaf beetle (*Oulema melanopa*)	Sobemovirus
Cocksfoot streak virus	CSV	W	Aphids (*M. persicae*, M*acrosiphum euphorbiae* and *Hyalopteroides humilis*)	Potyvirus
Cynosurus mottle virus	CnMoV	W & B	Cereal leaf beetle (*O. melanopa*)	Sobemovirus
European wheat striate mosaic virus	EWSMV	W & B	Planthoppers (*Javesella pellucida* and *J. dubia*)	Tenuivirus
Oat chlorotic stunt virus	OCSV	W & B	Unknown, possibly *P. graminis*	Tombusvirus
Oat mosaic virus	OMV	W	Soil-borne vector, *P. graminis*	Bymovirus
Ryegrass mosaic virus	RGMV	W	Mite (*Abacarus hystrix*)	Rymovirus
Soil-borne cereal mosaic virus	**SBCMV**	**W**	**Soil-borne vector, *P. graminis***	**Furovirus**
Wheat dwarf virus	**WDV**	**W & B**	**Planthoppers (primarily *Psammotettix alienus*)**	**Mastrevirus**
Wheat spindle streak mosaic virus	WSSMV	W	Soil-borne vector, *P. graminis*	Bymovirus

Agropyron mosaic virus (AgMV) is a *Rymovirus* affecting wheat and barley. It causes pale green to yellow mosaics, which can become less conspicuous with age (colouration and persistence of mosaic varies with cultivar), chlorosis and stunting. It also found in North America, Finland and Germany. The UK isolate is most closely related to a Canadian isolate (Flint, 2014). It is not considered to be of major economic importance, although yield reductions of up to 85% have been reported (Seifers, 1992).

Aubian wheat mosaic (AWMV) is a furo-like virus detected in France in 1999 that was found to be similar to a previously uncharacterised virus detected in Bedfordshire in 1995 in wheat (Clover *et al*., 1999a; Hariri *et al.*, 2001a). The “Bedford virus” caused faint mosaics in April followed by extensive chlorotic streaking in May and June, and resulted in yield reductions of 24% (Clover *et al*., 1999a). No further reports of this virus in the UK have been made.

Barley mild mosaic virus (BaMMV) is a *Bymovirus* affecting barley in Europe and Japan (Kühne, 2009). In the UK, barley mosaic viruses (including *Barley yellow mosaic virus*) were first detected in 1980 and have since been found across the country but is particularly severe in the East and the Cotswolds (Adams *et al.*, 1996). BaMMV was identified as the causal virus in 37% of plants exhibiting mosaic symptoms from 1987-90 (Adams, 1991). Yield losses can be up to 40% (Adams *et al*., 1996).

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BYDV is a *Luteovirus*, although Miller *et al.* (2002) contend that certain aspects of this virus suggest is a *Tombusvirus*. BYDV affects wheat and barley worldwide. It is the main virus of cereal crops in the UK, being estimated to affect 82% of winter wheat and 81% of winter barley (Clarke *et al.,* 2009). However, survey data has found large annual variation in incidence of the disease in the UK, with no detections in 2009 and 2011 (Flint, 2015) and 25% of wheat crops affected in 2017 (Judith Turner, Fera, pers. comm.). Several sub-groups of the virus are known globally but three predominate in the UK; RPV, MAV and PAV (Mann & Harrington, 1996). It should be noted that BYDV-RPV has since been reclassified as *Cereal yellow dwarf virus-RPV* (CYDV-RPV) based on differences in nucleotide sequences but is a relatively mild virus that is otherwise similar to BYDV (Miller *et al.*, 2002; Almasi *et al*., 2015). Flint's (2014) national survey of wheat viruses (1,356 samples across 4 years) found BYDV in 12 samples (6 each of MAV and PAV but none containing RPV). Infection affects yield formation in a number of ways, including reducing the number of plants per m^2, the number of tillers per plant, the number of grains per ear and thousand grain weight (McKirdy and Jones, 2002). The impact can be severe, with reported reductions in winter wheat yield of up to 2.1 t/ha (McKirdy & Jones, 2002) and 35% (Perry *et al.*, 2000) and in winter barley of up to 80% (Dedryver *et al*., 2010). However, much of the yield impact data originates from abroad and is lacking for UK.

Barley yellow mosaic virus (BaYMV) is a *Bymovirus* affecting barley in Europe and Asia (Kühne, 2009). The virus was first detected in the UK in 1980 but is likely to have been present for several years (Hill & Evans, 1980). A survey from 1987-90 found that over 70% of plants with mosaic symptoms had BaYMV (Adams, 1991). The virus is widespread in the UK but is particularly severe in the East and the Cotswolds (Adams *et al.*, 1996). Yield reductions of up to 50% have been reported (Plumb *et al.*, 1986).

Cocksfoot mild mosaic virus (CfMMV) is a *Sobemovirus* affecting wheat and barley in Europe and North America (Brunt et al. 1996). In the UK, CfMMV has been detected in other hosts but there have been no reports of natural infestations of wheat or barley (Flint, 2015). Light green streaks are symptomatic.

Cocksfoot mottle virus (CfMV) is a *Sobemovirus* affecting wheat and barley in Europe, Japan and New Zealand (Brunt et al. 1996). It has been detected in central and southern UK, causing green or chlorotic mottling, chlorotic streaking, whitening or chlorosis of older leaves, stunting and a reduction in tillering (Flint, 2014). If infected early, seedlings can die in 6-8 weeks (Serjeant, 1967). *Cocksfoot streak virus* (CSV) is a *Potyvirus* affecting wheat in Eurasia and North America (Brunt et al. 1996). It causes light or dark green streaking of leaves, reduced tillering and fewer fertile seeds (Flint, 2014).

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Cynosurus mottle virus (CnMoV) is a *Sobemovirus* affecting wheat and barley in Europe and New Zealand (Brunt et al. 1996). It has been detected in the UK since the mid-twentieth century (Brook, 1972). Symptoms include chlorotic mottling approximately one month after inoculation, followed by yellow streaking of the leaves (Flint, 2014). Experimental work has shown that the virus can result in a reduction in grain production of up to 83% (Flint, 2014). No information is available on the impact of the virus on barley.

European wheat striate mosaic virus (EWSMV) is a *Tenuivirus* affecting wheat and barley across Europe (Brunt et al. 1996). It causes fine, chlorotic striations of leaves followed by chlorosis, stunting and plant death (Flint, 2014). It has been estimated to reduce yield by up to 10% (Plumb, 1971).

Oat chlorotic stunt virus (OCSV) is a *Tombusvirus* affecting wheat and barley (Boonham *et al.*, 1997). The UK is the only country in which the virus has been reported (Flint, 2014). The virus causes conspicuous bright yellow streaking and severe stunting (Boonham *et al.*, 1997). Yield impact can be severe in oat (Boonham *et al.*, 1995) but it is thought to have less of an effect on wheat and barley (Boonham *et al.*, 1997).

Oat mosaic virus (OMV) is a B*ymovirus* affecting wheat in the UK and USA. Symptoms vary from eyespot-like symptoms (yellow or grey bordering a green area, especially in older leaves) to, less commonly, yellow or light green patches at the tips of the leaves (Flint, 2014). It is mainly a problem of oat but can infect wheat, although its impact on yield is unclear (McKinney, 1946).

Ryegrass mosaic virus (RGMV) is a *Rymovirus* affecting wheat in Eurasia, North America and Australia (Brunt et al. 1996). The British strain has not been reported to infect wheat but an American strain has been (Flint, 2014). It causes light green to chlorotic mosaics in ryegrass. Yield losses in rye can be high but its impact in wheat is unknown.

SBCMV is a *Furovirus* infecting wheat in Europe (Brunt *et al.* 1996). It is considered to be distinct from *Soil-borne wheat mosaic virus* present in Asia, North America and South America (Serfling *et al.*, 2009). In the UK, it was first detected in 1999 (Clover *et al*., 1999b) and has since been detected at other sites (Bass *et al.*, 2006; Flint, 2014). Yield losses due to the virus of up to 51% have been reported in the UK (Clover *et al*., 1999c). It is currently considered the second most important disease of cereals in the UK (Flint, 2014).

Wheat dwarf virus (WDV) is a *Mastrevirus* affecting wheat and barley. It is widely distributed in Europe and is also present in Asia (Serfling *et al.*, 2016). Few reports of WDV exist in the UK but it has been detected in Cambridgeshire in 2018 and 2019 (KWS, pers. comm.). In Europe, it is a serious but sporadic problem (Lindblad & Waern, 2002), capable of causing

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100% reductions in yield (Širlová *et al.*, 2005). Several strains have been identified but these tend to primarily infect either wheat or barley (Koklu *et al*., 2007; Kis *et al*., 2019). *Wheat spindle streak mosaic virus* (WSSMV) is a *Bymovirus* affecting wheat in North America, South Asia, East Asia and Europe (Brunt et al. 1996). It was first recorded in the UK in 1968 but was not detected in national surveys of cereal viruses between 2009 and 2012 (Flint, 2015). It causes stunting and reduced tillering (Budge *et al.*, 2008b). Yield reductions of 32% have been reported in the USA (Miller *et al*., 1992) but no serious problems have been reported so far in the UK.

New emerging viruses

In 2011, next generation sequencing was used to look for novel viruses in a wheat crop in Suffolk, UK (Flint, 2015). This found several potentially novel viruses, including one that appeared in a quarter of all samples. None of the plants exhibited any symptoms suggesting that these novel viruses may be cryptic. Globally, wheat and barley are susceptible to 58 and 57 viruses respectively but many of these viruses have not yet been detected in the UK (Brunt et al. 1996) (Flint, 2014). However, only a handful are considered economically important (Serfling *et al.*, 2016) (Table 6).

Table 6. Summary of viruses reported from wheat and barley not currently reported in the UK that cause significant yield losses elsewhere in the world. W = wheat, B = barley.

Virus	**Acronym**	**Crop**	**Genus**
Barley yellow dwarf virus (non-UK isolates)	BYDV	W & B	*Luteovirus*
Soil-borne wheat mosaic virus	SBWMV	W & B	*Furovirus*
Wheat streak mosaic virus	WSMV	W	*Tritimovirus*
Wheat spindle streak mosaic virus (non-UK isolates)	WSSMV	W	*Bymovirus*
Wheat yellow mosaic virus	WYMV	W	*Bymovirus*

BYDV. There are several subgroups and isolates of BYDV that are not currently present in the UK, e.g. BYDV-PAS, -RPS, -SGV and –kerII (Serfling *et al.*, 2016). These include CYDV-RPS (formerly CYDV-RPV-Mex1), which causes severe stunting, leaf rolling and notching (Almasi *et al*., 2015) and PAV-129, a resistance breaking isolate (Chay *et al*., 1996). The arrival of these subgroups into the country presents an additional risk to cereal production and could occur either by importation (via infected vectors or plant material) or by an increase in the geographic range of vectors.

Soil-borne wheat mosaic virus (SBWMV) is a *Furovirus* affecting wheat and barley. It is found in the USA (Putman *et al*., 1994), South America, East Asia and Germany (Ziegler *et al*., 2013). Yield reductions of 50% have been reported (Drumm Myers *et al.,* 1993).

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Wheat streak mosaic virus (WSMV) is a *Tritimovirus* affecting wheat in the Americas, Europe, the Middle East and Australasia (Singh *et al.*, 2018). It is considered to the one of the most important factors limiting grain production in some regions (Workneh *et al.*, 2009) and an increasing issue worldwide (Singh *et al.*, 2018). Yield impacts of up to 76% have been reported (Velandia *et al.*, 2010).

WSMV has already been detected in the UK with few issues but strains present in other countries cause significant yield losses. In the USA, yield reductions of up to 59% have been reported (Slykhuis, 1970), and in Italy, where the virus occurred in a mixed infection with SBCMV, yield losses reached 70% (Vallega and Rubies-Autonell, 1985). The arrival of such strains in the UK may increase the importance of this virus.

Wheat yellow mosaic virus (WYMV) is a *Bymovirus* found in East Asia, Canada and Europe (Brunt et al. 1996). It can result in yield reductions of up to 70% (Serfling *et al.*, 2016).

The rest of this review of cereal viruses will focus on the viruses highlighted in this section (non-UK subgroups of BYDV, SBWMV, WSMV, non-UK isolates of WSSMV and WYMV) along with those identified in Table 5.

Vectors of key viruses

Table 7. Vectors of key viruses of cereals. Main vector(s) is highlighted. Note only vectors of UK BYDV isolates are shown here. Non-UK BYDV vectors are discussed in the text.

Virus	**Vector**	
	Latin name	Common name (where applicable)
Barley mild mosaic virus	***Polymyxa graminis***	n/a
Barley yellow dwarf virus-MAV	***Sitobion avenae****, Rhopalosiphum padi*, *Metopolophium dirhodum and R. maidis*	**Grain aphid**, bird cherry-oat aphid, rose-grain aphid and corn leaf aphid
Barley yellow dwarf virus-PAV	***S. avenae****,* ***R. padi***, *M. dirhodum and R. maidis*	**Grain aphid**, **bird cherry-oat aphid**, rose-grain aphid and corn leaf aphid
Barley yellow dwarf virus-RPV	***R. padi****, S. avenae* and *R. maidis*	**Bird cherry-oat aphid**, grain aphid and corn leaf aphid
Barley yellow mosaic virus	***Polymyxa graminis***	n/a
Soil-borne cereal mosaic virus	***Polymyxa graminis***	n/a
Soil-borne wheat mosaic virus	***Polymyxa graminis***	n/a
Wheat dwarf virus	***Psammotettix alienus****, P. provincialis*	n/a

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Wheat streak mosaic virus	***Aceria tosichella***	**Wheat leaf curl mite**
Wheat spindle streak mosaic virus	***Polymyxa graminis***	n/a
Wheat yellow mosaic virus	***Polymyxa graminis***	n/a

BaMMV: The soil inhabiting plasmodiophorid, *Polymyxa graminis*, transmits BaMMV (Adams, 1990). *P. graminis* has been recorded around the world, including the UK (CABI, 2011) and transmits several viruses (see Table 7), with different isolates of the plasmodiophorid transmitting different viruses (Adams, 1990). Infection is initiated when motile zoospores penetrate epidermal or root hair cells. The zoospore then undergoes reproduction within the host, eventually forming a multinucleate sporangial plasmodium, which is separated from the host by a cell wall. This structure subsequently produces new zoospores, which either cause fresh infections (thereby repeating the same cycle) or form into a sporogenic plasmodia, which in turn develops into resting spores (Kanyuka *et al.*, 2003). The resting spores remain viable in the soil for several decades (Kanyuka *et al.*, 2003). The means by which *P. graminis* acquires a virus and transfers it to the crop is poorly understood, but it is thought to occur either during the initial infection of the epidermal or root hair cell or during the sporogenic plasmodia stage (Kanyuka *et al.*, 2003). Whether the virus can multiply in *P. graminis* is also unknown but it is thought to be unlikely (Kanyuka *et al.*, 2003). Environmental conditions can affect the activity and population increase of *P. graminis*, although this is also affected by the provenance of the *P. graminis*. For instance, the development of an isolate from a temperate region occurred slowly at 10°C, most quickly at 15-22°C but with almost no development above 20-22°C (Slykhuis & Barr, 1978; Adams & Swaby, 1988; Legrève *et al.*, 1998). Whereas the optimal temperature for development of an Indian isolate was 27-30°C, with little development at 19-22°C (Legrève *et al.*, 1998). BaMMV can also be transmitted mechanically (Timpe & Kühne, 1995).

BYDV: Several aphid species circulatively and persistently transmit BYDV, with different BYDV subgroups vectored by specific aphid species. The vectors for the UK BYDV subgroups are (Rochow, 1969; Dewar *et al*., 2016):

MAV: Primarily the grain aphid (*S. avenae*) and rarely bird cherry-oat aphid (*R. padi*), rose-grain aphid (*Metopolophium dirhodum*) and corn leaf aphid (*R. maidis*).

PAV: Primarily *S. avenae* and *R. padi*, and rarely *M. dirhodum* and *R. maidis*.

RPV: Primarily *R. padi*, and rarely *S. avenae* and *R. maidis*.

For the disease generally, *S. avenae* is considered to be the most important vector in northern England and Scotland, and *R. padi* the most important in southern England and Wales

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(McGrath & Bale, 1989). Both species migrate into winter cereals in the autumn and can spread infections to spring cereals in the spring. Mild autumn and winter weather increases disease risk by lengthening the period of migration into crops in the autumn and increasing within-crop spread in the winter (Lowles *et al.* 1997). Apterous (wingless) *R. padi* have also been shown to preferentially immigrate to BYDV-infected wheat over non-infected wheat, an adaptation that may accelerate BYDV spread (Jimenez-Martinez *et al*., 2004; Medina-Ortega *et al*., 2009).

Non-UK BYDV subgroups are often transmitted by aphid species that do not occur, or are not common, in the UK. For example, BYDV-SGV is transmitted by the aphid, *Schizaphis graminum*, which is common in southern Europe but has not been found on UK crops. However, if weather conditions become warmer in northern Europe it is possible that the range of *S. graminum* would increase to include the UK (Kati *et al.*, 2013). Similarly, BYDV-PAS, which has been reported to be more severe than BYDV-PAV in barley (Chay *et al*., 1996), is vectored by the aphids *R. maidis* and *M. dirhodum* in central Europe (Jarosõvá *et al*., 2013), and while both species are already present in the UK, their activity could increase if temperatures in the UK, especially in the winter, become warmer (Capinera, 2008; Honek *et al.*, 2018).

BaYMV: This virus is spread by *P. graminis* (Adams *et al*., 1988; Laing 1989), although it is thought that only a small proportion of spores carry the virus (Adams *et al*., 1988). See the section on BaMMV transmission above for further information on *P. graminis*. Plants can also be infected by mechanical transmission (Adams *et al*., 1988).

SBCMV: *P. graminis* is the vector for SBCMV (Ratti *et al*., 2004). The virus is able to survive in *P. graminis* resting spores in the absence of wheat for at least 15 years (Bayles *et al.*, 2007). See the section on BaMMV transmission above for further information on *P. graminis*. It can also be transmitted by seed (Clover *et al*., 1999a), with a transmission rate of up to 9.4% in wheat (Budge *et al.*, 2008a).

SBWMV: *P. graminis* is the vector for SBWMV (Rao, 1968). See the section on BaMMV transmission above for further information on *P. graminis*. There is some suggestion that SBWMV can also be transmitted by seed (Jeżewska *et al.*, 2016).

WDV: This virus is transmitted by leafhoppers in a semi-persistent, non-propagative manner. *Psammotettix alienus* is the considered the main vector in Europe (Vacke, 1961) and China (Wang *et al*., 2014) but *P. provincialis* has been identified as the vector in Syria (Ekzayez *et al*., 2011). *P. alienus* is able to transmit the virus to healthy plants within five minutes of acquiring the virus (Wang *et al*., 2014). In Sweden, *P. alienus* is commonly found in crops and grasslands (Lindblad & Waern, 2002) but in the UK it is rare (Defra, 2015). In mainland

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Europe, initial infection occurs in the late summer when the leafhopper migrates into winter crops (Lindblad & Waern, 2002; Širlová *et al.*, 2005). Adult *P. alienus* remain active into the autumn, providing secondary spread of the virus (Manurung *et al*., 2004; Manurung *et al*., 2005). In the spring, further secondary spread occurs when newly hatched nymphs acquire the virus from plants already infected (Lindblad & Waern, 2002). Defra (2015) concluded that both natural spread of the virus to the UK and its importation is unlikely because *P. alienus* is not thought to migrate long distances and there have been no reports of *P. alienus* in ornamental grass nurseries or WDV in ornamental grass species. The reason for the relative rareness of *P. alienus* in the UK is not obvious (Defra, 2015). Roos *et al.* (2011) found that cool autumns in Sweden decrease activity and suggest that climate warming may increase problems with the pest and virus. Climate change may also increase the range of the pest to the UK.

WSMV: The wheat leaf curl mite, *Aceria tosichella*, transmits WSMV (Harvey & Seifers, 1991). This mite is widespread in North America and present in South America, Asia, Europe and Australia (Cabi, 2018). *A*. *tosichella* is present in the UK (Chamberlain & Evans, 1980) but it is not common nor is it considered a pest of cereals. In the USA, where the pest and WSMV are major problems, populations of *A*. *tosichella* build up on non-crop hosts and volunteers during the summer before being carried by the wind into winter cereals (McMechan, 2016). Early infestations with *A*. *tosichella* resulted in greater WSMV infections and yield reductions (Wosula *et al.*, 2017). *A*. *tosichella* population increase is greatest above 25°C and arrested below 15°C (McMechan, 2016). Ranabhat *et al.* (2018) found that *A*. *tosichella* infestations and WSMV infections were highest where mean temperature in October was above 15°C and the crop was bordered by *Bromus tectorum* and pre-harvest volunteer wheat. As temperature dropped so did WSMV infections (Ranabhat *et al.*, 2018). In the UK, the importance of *A*. *tosichella* is likely limited by relatively cooler and wetter summer conditions, average October temperatures being below 13°C for the majority of the country (Met Office, 2019) and the rarity of *B. tectorum* (NHM, 2018). In Europe, it has been suggested that climate change-induced increases in temperatures could result in *A*. *tosichella* becoming important in new regions (Skoracka *et al.*, 2017). *A*. *tosichella* and its importance as a viral vector is further reviewed in Skoracka *et al.* (2018). WSMV can also be transmitted by seed, with a transmission rate between 0.2-1.5% (Jones *et al*., 2005; Roger *et al*., 2005, Lanoiselet *et al*., 2008).

WSSMV: *P. graminis* is widely considered to be the vector for WSSMV (Slykhuis & Barr, 1978), however there remains some doubt in this regard (Cadle-Davidson & Bergstrom, 2014). Transmission by *P. graminis* is thought to be slow and to occur at a limited temperature range (Slykhuis & Barr, 1978). The ideal temperature for transmission of the

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virus is 15°C and for development of the virus is 10°C (Slykhuis & Barr, 1978). Leaf symptoms disappear above 17°C (Serfling *et al*., 2016). However, Cadle-Davidson & Bergstrom (2014) found that environmental conditions conducive to WSSMV infection differed to those for another *P. graminis* vectored virus, SBWMV. See the section on BaMMV transmission above for further information on *P. graminis*. The virus can be transmitted mechanically but plant susceptibility to this method of transmission is low (Slykhuis, 1975).

WYMV: *P. graminis* is the vector for WYMV (Hariri *et al*., 1987). See the section on BaMMV transmission above for further information on *P. graminis*.

Alternate host species of key viruses

The main alternative hosts in the UK of the key wheat and barley viruses are given in Table 8. The presence of the alternate hosts in the UK was checked using the Online Atlas of the British and Irish flora (BRC, 2019).

Table 8. Alternate UK host species for the key wheat and barley viruses.

Virus	**Alternate host**
BaMMV	*Lagurus ovatus*
BYDV	*Anthoxanthum odoratum, Avena spp., Bromus spp., Dactylis glomerata, Festuca pratensis, Lolium spp. Panicum dichotomiflorum, Phalaris arundinacea, Phleum pratense, Poa spp. Secale cereale, Zea mays*
BaYMV	None
SBCMV	*Bromus commutatus, Secale cereale*
SBWMV	*Bromus commutatus, Secale cereale*
WDV	*Avena spp., Bromus secalinus, Lagurus ovatus, Lolium spp., P. annua, S. cereale*
WSMV	*Avena sativa, Bromus* spp., *Digitaria* spp., *Echinochloa* spp., *Eragrostis* spp., *Hordeum* spp., *Lolium* spp., *Panicum* spp., *Phalaris* spp., *Poa compressa, Poa* spp., *Secale cereale, Setaria viridis*, *S. faberi, Stipa* spp., *Zea mays*
WSSMV	*Secale cereale*
WYMV	*Secale cereale*

Detection and Identification of Viruses

Early Detection, Surveillance, and Management of Viruses

A variety of diagnostic methods have been developed for the detection and identification of wheat and barley viruses. These are listed in Table 9.

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Table 9. Methods for detection and identification of key cereal viruses.

Relevant virus	Detection method
BaMMV	Symptomatology, ELISA, immunospecific electron microscopy (ISEM), Western blot, coat protein sequencing, Southern blot, PCR, RT-PCR, multiplex nested PCR, RFLP
BYDV	Symptomatology, ELISA, electron microscopy, ISEM, Western blot, coat protein sequencing, precipitation tests, agglutination tests, transmission tests, Southern blot, PCR, RT-PCR, qRT-PCR, multiplex nested PCR, RFLP, LAMP, aerial imagery, spectral imaging
BaYMV	Symptomatology, ELISA, electron microscopy, ISEM, Western blot, coat protein sequencing, Northern blot, PCR, RT-PCR, qRT-PCR, multiplex nested PCR, RFLP
SBCMV	Symptomatology, ELISA, electron microscopy, ISEM, monoclonal antibodies, Western blot, PCR, RT-PCR, qRT-PCR
SBWMV	Symptomatology, ELISA, electron microscopy, ISEM, monoclonal antibodies, Western blot, coat protein sequencing, RT-PCR, multiplex nested PCR, LAMP
WDV	Symptomatology, ELISA, electron microscopy, ISEM, Western blot, transmission tests, PCR, RT-PCR, multiplex nested PCR, RFLP, aerial imagery
WSMV	Symptomatology, ELISA, electron microscopy, ISEM, Western blot, immunoblotting, protein fingerprinting, PCR, RT-PCR, multiplex nested PCR, RFLP, LAMP, aerial imagery, hand-held radiometer
WSSMV	Symptomatology, ELISA, ISEM, Western blot, Northern blot, RT-PCR, qRT-PCR
WYMV	Symptomatology, ELISA, electron microscopy, ISEM, Western blot, coat protein sequencing, RT-PCR, multiplex nested PCR, RFLP, LAMP

Detection and Identification Methods Based on Biological Properties

BaMMV: Symptoms include patches of plants developing yellow mosaic flecking and pale yellow longitudinal streaks, which may eventually form necrotic patches, and leaves can roll to give a spiky appearance (Adams *et al.*, 1996). Symptomatology can be useful in diagnosing the virus but is similar to those for BaYMV (Huth & Adams, 1990). The virus particles are flexous and rod-shaped, with two modal lengths of 500-600 nm and 250-300 nm (Kashiwazaki & Hibino, 1996). Identification by ELISA (Adams, 1991; Nomura *et al*., 1996), immunospecific electron microscopy (ISEM) (Adams, 1991) and Western blot (Dessens & Meyer, 1995) has been developed. The amino acid sequence of the viral coat protein has been determined (Foulds *et al*., 1993).

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BYDV: Symptoms usually involve stunting, floret blasting (especially in oats) and foliar water soaking and chlorosis (blotches, stripes or mottling) beginning at the leaf tip (Rochow, 1969). Chlorosis tends to be chrome-yellow in barley, red-yellow in wheat and red-purple in oats. Symptoms are more severe in oats than barley, and least severe in wheat. Development of symptoms (for symptomology testing) often need cool temperatures and supplementary lighting in a greenhouse (Rochow, 1969). Electron microscopy has been used to describe the virus (Gill & Chong, 1970), with an isometric virion, 25 nm in diameter and not enveloped. Detection and identification of the PAV, MAV and RPV strains in plant material using ELISA (Barbara & Clark, 1982; Clement *et al*., 1986; Lister & Rochow, 1979) and ISEM (Forde, 1989) has been demonstrated. ELI4SA has also been developed to detect BYDV in aphid vectors (Torrance *et al.*, 1987). Detection using Western blot analysis has also been demonstrated (Vincent *et al*., 1991). The BYDV coat protein sequence has been described, including for the PAV (Miller *et al*., 1988; Vincent *et al*., 1990), MAV (Vincent *et al*., 1990) and RPV (Vincent *et al*., 1990) strains. Purification via precipitation has shown promise (Rochow, 1970). Viral strains can be discerned using a latex agglutination test (Aapola & Rochow, 1971). As specific aphid species transmit different strains of BYDV, these can be used to assist in identifying the virus and strain (Rochow, 1969).

BaYMV: The virus causes irregular chlorotic streaking along leaf veins, which are most distinct on young foliage and can range from pale yellow to orange-yellow, and upward rolling of leaf margins (Friedt, 1983). Symptoms can assist in diagnosis but are similar to BaMMV (Huth & Adams, 1990). The virion is filamentous, not enveloped and often flexuous, with two modal lengths of 270-290 nm and 570-600 nm (Brunt et al. 1996). The amino acid sequence of the viral coat protein has been determined (Kashiwazaki *et al*., 1989). Detection using ELISA (Usugi *et al*., 1984; Adam, 1991; Hariri *et al*., 1996a; Hariri *et al*., 2008), Western blot (Sohn *et al*., 1995; Hariri *et al*., 1996a; Hariri *et al*., 2008), electron microscopy (Kashiwazaki *et al*., 1989) and ISEM (Adams, 1991) has been demonstrated.

SBCMV: Symptoms include faint pale green to yellow mosaics during April, developing into chlorotic streaks in May, stunting and a reduction in both tillering and grain yield (Clover *et al*., 1999a; Budge *et al.*, 2008b). Symptoms can be used to assist in identification but are similar to those for SBWMV and WSSMV (Budge *et al.*, 2008b). The virus has been described using electron microscopy (Clover *et al*., 1999a). The virus particle has a stiff, rod shape and is 18-20 nm wide by 100, 220 or 300 nm long (Clover *et al*., 1999a; Serfling *et al*., 2016). ELISA tests have been developed for SBCMV but these are often poor at discriminating between the closely related SBWMV (Clover *et al*., 1999c; Clover *et al*., 2001), however monoclonal antibodies able to discriminate between German strains of SBCMV and SBWMV have been produced (Rabenstein *et al*., 2005). Detection using ISEM has been demonstrated

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(Vallega *et al*., 2006). Western blot can be used to detect the virus but cannot discriminate between it and SBWMV (Rabenstein *et al*., 2005). Other methods developed for the identification of SBWMV may be effective for SBCMV (see SBWMV section below).

SBWMV: Symptoms include severe rosetting, arrested spring development, excessive tillering, stunted roots and light green to yellow foliar mosaics (irregular steaks along the long axis of the leaf) that are mainly seen in early spring (Smith, 1972; Brunt et al. *et al.*, 1996). Symptoms are similar to SBCMV but can be used to discriminate from other viruses. Electron microscopy has been used to describe the virion and its inclusion bodies (Peterson, 1970; Hibino *et al.*, 1974; Littlefield, 2003). The virion is a hollow rod-shape 20 nm wide. There are at least two components; the larger is 281-300 nm and the smaller is 138-160 nm or 92-110 nm long (Shirako & Brakke, 1984). Diagnosis of SBWMV has been developed with ELISA (Usugi *et al*., 1984; Bahrani *et al*., 1988; Chen *et al*., 1997) and Western blot (Ohsato *et al*., 2003). The viral coat protein has been described and detection using ISEM demonstrated (Chen *et al*., 1997).

WDV: Symptoms include severe dwarfing and foliar chlorosis, reddening and streaking (Širlová *et al.*, 2005). The virus has been described using electron microscopy (Suärez-López *et al* 1995). The virion is arranged in a pair (or geminate) not enveloped and 18 nm by 30 nm long (Brunt et al. 1996). ELISA (Ramsell *et al*., 2008; Zhang *et al*., 2018), ISEM (Roberts *et al*., 1984), Western blot (Liu *et al* 2014) and transmission tests (Vacke, 1961; Ekzayez *et al*., 2011) have been used to detect the WDV in plant material. ELISA has also been developed to detect the virus in *P. alienus* (Vacke & Cibulka, 2000).

WSMV: Symptomology includes faint chlorotic streaks parallel to the leaf veins, mottling, stunting, tillers of uneven height, sterile heads and reduced grain fill (Smith, 1972). Virions are flexuous rods 700 nm in length (Smith, 1972). Detection of WSMV in plant material has been demonstrated using ELISA (Sherwood, 1987; Montana *et al*., 1996), Western blot (Sherwood, 1987; Montana *et al*., 1996), immunoblotting (Choi *et al.*, 1999), protein fingerprinting (Montana *et al*., 1996), electron microscopy (Montana *et al*., 1996) and ISEM (Foulad & Izedpanah, 1986). Detection of viruliferous *A. tosichella* has been developed using immunofluorescent microscopy and dot-immunobinding assays (Mahmood *et al.*, 1997).

WSSMV: Symptoms of the disease include young leaves producing light green to yellow short streaks and spindle-shaped dashes, while older foliage exhibits mosaics and necrosis (Smith, 1972). Virions are filamentous and 700 nm long (Brunt et al. 1996). The use of ELISA to detect WSSMV has been demonstrated (Bays *et al*., 1986; Zagula *et al*., 1990), although the analysis can struggle to discriminate between this virus and both WYMV and BaYMV (Hariri *et al*., 1996a; Marie-Jeanne *et al*., 1999). Western blot has also been used to identify

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the virus (Sohn *et al*., 1995) but failed to discriminate it from BaYMV and WYMV (Marie-Jeanne *et al*., 1999). ISEM has also been used for identification (Baker *et al*., 1985) but could not differentiate the virus from WYMV (Marie-Jeanne *et al*., 1999).

WYMV: Symptoms include dwarfing and irregular foliar chlorotic streaks and yellowing (Serfling *et al*., 2016). Virions are not enveloped, filamentous, often flexuous and 13-14 nm wide by either 275-300 nm or 575-500 nm long (Brunt et al. 1996). ELISA has been developed for identification of the virus (Usugi *et al*., 1984; Kusume *et al*., 1997) but can struggle to discriminate between it and both WSSMV and BaYMV (Hariri *et al*., 1996b). Detection with Western blot has also been developed (Xing *et al*. 2000; Dong *et al*., 2002; Fukuta *et al*., 2013). ISEM has been shown to identify the virus but was unable to differentiate between WYMV and WSSMV (Han *et al.*, 2000). The coat protein sequence has been described and could be used for identification (Han *et al.*, 2000). Viral morphology and its effects on the host cell has been described using electron microscopy (Hibino *et al*., 1981; Xie *et al*., 2019).

Detection and Identification Methods Based on Viral Nucleic Acid

BaMMV: Detection of BaMMV has been demonstrated using Southern blot (Pröls *et al*., 1990; Andersen, 1991), PCR, (Dessens & Meyer, 1995); RT-PCR (Kashiwazaki & Hibino, 1996; Rha, 2001; Mumford *et al*., 2004), multiplex nested PCR (Mumford *et al*., 2004) and RFLP (Dessens & Meyer, 1995).

BYDV: Detection of the UK BYDV isolates in plant material has been demonstrated using Southern blot (Waterhouse *et al*., 1986; Vincent *et al*., 1990), PCR (Robertson *et al*., 1991), RT-PCR (Malmstrom & Shu, 2004; Chomič *et al*., 2010) and qRT-PCR (Flint, 2015). RT-PCR has been developed to detect the virus in aphid vectors (Canning *et al*., 1996; Fabre *et al.*, 2003b). RFLP analysis has been demonstrated for some isolates (Moon *et al*., 2000; Kundu *et al*., 2009). Multiplex nested PCR has been demonstrated for Chinese isolates (including PAV) (Tao *et al*., 2012). Relatively low cost and highly sensitive detection of USA (Deb & Anderson, 2008) and Chinese isolates (including PAV) with LAMP has been demonstrated (Zhao *et al*., 2010).

BaYMV: PCR (Dessens & Meyer, 1995), multiplex nested PCR (Mumford *et al*., 2004), RFLP (Dessens & Meyer, 1995), Northern blot and RT-PCR (Sohn *et al*., 1995; Shi *et al*., 1996; Mumford *et al*., 2004) diagnostics have been developed for BaYMV.

SBCMV: Detection of SBCMV has been demonstrated with PCR (Ratti *et al*., 2004), RT-PCR (Clover *et al*., 1999c; Ratti *et al*., 2004; Vaïanopoulos *et al*., 2009) and qRT-PCR (Ziegler *et al*., 2014; Flint, 2015).

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SBWMV: RT-PCR (Gitton *et al.*, 1999), multiplex nested PCR (Deb & Anderson, 2008) and LAMP (Fukuta *et al*., 2013) detection methods have been developed for SBWMV.

WDV: Detection of this virus has been demonstrated using PCR (MacDowell *et al*., 1985; Padidam *et al*., 1995), RT-PCR (Zhang *et al*., 2010; Gadiou *et al*., 2012), multiplex nested PCR (Tao *et al*., 2012) and RFLP (Schubert *et al*., 2005; Kundu *et al*., 2007). RT-PCR has also been developed to detect the virus in the vector, *P. alienus* (Zhang *et al.*, 2010).

WSMV: Detection of WSMV in plant material has been demonstrated using PCR (French & Robertson, 1994), RT-PCR (McNeil *et al*., 1996), multiplex nested PCR (Deb & Anderson, 2008), RFLP (McNeil *et al*., 1996) and LAMP (Lee *et al*., 2015). Detection of WSMV in *A. tosichella* has been demonstrated with nucleic acid elution methods (Price *et al.*, 2014).

WSSMV: Northern blot (Sohn *et al*., 1995), RT-PCR (Sohn *et al*., 1995), multiplex nested PCR (Gitton *et al*., 1999; Deb & Anderson, 2008) and qRT-PCR (Ziegler *et al*., 2014; Flint, 2015) methods of detecting WSSMV have been developed.

WYMV: Detection of the virus has been demonstrated using RT-PCR (Han *et al.*, 2000), multiplex nested PCR (Clover & Henry, 1999; Deb & Anderson, 2008; Tao *et al*., 2012), RFLP (Ohki *et al*., 2014) and LAMP (Zhang *et al*., 2011; Fukuta *et al*., 2013).

Novel technologies

Aerial photography has been found to be capable of detecting plants infected with BYDV (Greaves *et al.*, 1983; Gaboijanyi *et al*., 2003) and WDV (Gaboijanyi *et al*., 2003). However, this detection method was unable to discriminate between BYDV and WDV infections (Gaboijanyi *et al*., 2003) and is unlikely to be effective before visual disease symptoms appear, i.e. when timely vector management decisions would be made. Using spectral imaging equipment to take readings approx. 0.75 m above the crop at GS25, Mirik *et al*. (2006) were able to identify relationships between spectral vegetation indices and both aphid density and damage on winter wheat, particularly when using hyperspectral imagery. This presents the potential to monitor and quantify aphid infestation in wheat under growing conditions, although this work involved only one of the UK's main BYDV vectors, *R. padi*. If this approach were found to be similarly effective at monitoring aphid infestations of both *R. padi* and *S. avenae* at earlier growth stages then this approach could be useful for informing treatment decisions in the autumn and early winter in the UK.

Remote sensing of WSMV using a hand-held radiometer (Workneh *et al*., 2009), satellite (Kanemasu *et al.*, 1974; Mirik *et al*., 2011; Mirik *et al*., 2013) and aerial imagery (Stilwell *et al*., 2019) has been shown to be effective at discriminating (WSMV) diseased and healthy wheat. Satellite imagery presents a useful and inexpensive means of identifying and

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"mapping disease incidence over large and remote areas" (Mirik *et al*., 2011) and "may substantially improve monitoring, planning, and [WSMV] management practices" (Mirik *et al*., 2013). No remote sensing literature was found for the other key cereal viruses nor any literature on novel robotics-type technology for any of the key cereal viruses.

Modelling

Several decision support systems (DSS) have been developed for control of BYDV (e.g. Kendall *et al*., 1992; Tatchell *et al*., 1994; Morgan, 2000; Fabre *et al.*, 2003a; Foster *et al*., 2004; HGCA, 2004; Fabre *et al.*, 2006; Thackray *et al*., 2009; Walls *et al*., 2016; Enders *et al*., 2018). These help determine risk and provide growers and advisors with advice on control measures such as seed treatments, foliar insecticides and cultural control. Nevertheless, DSS for virus management are underused in the UK for several reasons, including poor performance (Rose *et al*., 2016), lack of computational power (Harrington *et al.*, 1999) and the availability of cheap insecticides (e.g. pyrethroids) that make crop monitoring uneconomic (Ellis *et al*., 2009). However, the power of modern personal computers and phones mean that even moderately power-intensive DSS should be accessible to most users.

Data on the proportion of aphids carrying the virus has been suggested as an important means of improving DSS (Harrington *et al.*, 1999; Dedryver *et al.*, 2010). *SBWMV*: A model has been developed to predict post-planting infection based on soil temperature and water availability (Cadle-Davidson *et al*., 2003) and has been validated in-field (Cadle-Davidson & Bergstrom, 2014). The authors suggested that planting date is less important for determining virus pressure than post-planting environmental conditions and that the model provides the potential to determine yield losses and assist in crop management decisions (Cadle-Davidson & Bergstrom, 2014).

Lindblad & Arenö (2002) investigated the effect of cropping practices and weather on the vector of WDV, identifying the importance of late sowing and cool autumn conditions for limiting virus risk.

Ranabhat *et al.* (2018) used modelling approaches to identify risk factors for WSMV and its vector, *A. tosichella*. They found that warm mean daily temperatures (>10°C), the presence of *B. tectorum* around the crop and pre-harvest volunteer wheat significantly increased the risk of virus infection, while bare ground around the crop and lower mean temperatures resulted in low levels of virus risk. Workneh *et al*. (2009) developed a model to relate hand-held radiometer measurements of disease in the spring with yield impact across a field. Workneh *et al*. (2017) further developed the model to predict yield losses based on spring disease severity to assist in assessing the economic benefits of crop management decisions. The spatial dynamics of *A. tosichella* movement and virus spread were modelled by Stilwell

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et al. (2019), providing the potential to assist in crop management but further work is needed to refine risk factors. A day degree model has also been developed to help predict virus detection and so improve sampling and testing effort and reduce costs (Burrows *et al*., 2016). *WSSMV*: Some work has been conducted to identify risk factors for the virus (Cadle-Davidson & Bergstrom, 2014).

Chemical and cultural management options

This section will describe virus management for the UK viruses identified inthe Current viruses present in the UK section above.

BaMMV: Reduction of populations of the vector, *P. graminis*, using soil fumigants has been demonstrated but these rarely penetrate deep enough to provide effective control, are often impractical and ecologically unacceptable (Kühne, 2009; Roberts, 2014). This means that the primary method of managing BaMMV is through the use of resistant cultivars (Kühne, 2009). Resistant cultivars are commercially available (see Breeding for aphid resistance and host resistance and/or tolerance section below) and all varieties in the 2019 RL list in the UK are resistant (AHDB, 2019). However, the use of resistant cultivars does not necessarily reduce viral pressure in following seasons, Adams *et al.* (1993) found no significant reduction *P. graminis* populations (viruliferous or non-viruliferous), disease symptoms or yield impacts in susceptible crops after growing resistant varieties for three years on land naturally infested with BaMMV. Crop rotation is not an effective method of control as viruliferous *P. graminis* can remain in the soil for up to eight years (Kanyuka *et al.*, 2003). Preventing the movement of soil from infected to uninfected sites and cleaning equipment with anti-viral agents will minimise the viral spread, however such activities rarely occur due to financial and time constraints (Flint, 2015).

BYDV: Chemical control of the aphid vectors of BYDV is the most commonly used method of combatting BYDV in the UK. Neonicotinoid seed treatments that provide 4-6 weeks control of aphids post-emergence have been widely used in cereals for several years, however European Commission restrictions on these treatments means that these will not be available from 2019. Foliar pyrethroids (alpha-cypermethrin, beta-cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, lambda-cyhalothrin, tau-fluvalinate and zeta-cypermethrin) are currently the only actives registered for control of BYDV aphids in the autumn in the UK. However, *S. avenae* with knockdown (kdr) resistance is widespread in the UK (IRAG, 2019b). This resistance confers reduced sensitivity to pyrethroid insecticides, meaning that pyrethroids may not provide effective control. IRAG guidance (2019b) is that “pyrethroid treatments must be applied at the full recommended rate to maximise the effectiveness of the treatment and to reduce the chances of grain aphid [*S. avenae*] evolving stronger pyrethroid

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resistance. It is also important to ensure good crop coverage, because pyrethroids only have contact activity against aphids". There is no evidence of pyrethroid resistance in other UK BYDV vectors (IRAG, 2019b).

The general advice on control of BYDV is to monitor pests, particularly during the period in which the crop is vulnerable and during the early stages of infestation. Monitoring should continue for as long as conditions remain conducive for aphid migration (IRAG, 2019a). For example, in the autumn aphids will fly if temperatures are above 15°C but continue to move within crops at relatively low temperatures. It is hard to be precise about the level of frost needed to deliver a knock-out blow but three to five consecutive days with grass minima dropping below -6°C should cause high mortality. Monitoring tools can be used to help time crop inspections for aphids such as those available from the Rothamsted Insect Survey and AHDB Aphid News.

To maximise BYDV control with pyrethroids it is important to ensure sprays are timed correctly. For example, tank mixes of herbicides and insecticides are sometimes applied at the optimum time for weed control but not aphid control, which can result in poor aphid control (IRAG, 2019b). BYDV control is optimal when foliar insecticides are applied to target the second generation of wingless aphids in the crop. This is because the initial aphid infestation affects relatively few plants and it is the second generation of wingless aphids that tend to move away from these originally colonised plants (IRAG, 2019b). The timing of the second generation can be approximated by accumulating daily average air temperatures above a baseline temperature of 3ºC. The second generation takes around 170 'day degrees' (DD) to be produced (IRAG, 2019b). A tool to calculate when the 170 DD threshold is reached, and that takes into account the region, crop emergence date and insecticide use, is available on the AHDB website (https://cereals.ahdb.org.uk/bydv). Similarly, BYDV Assist is a freely available mobile app developed by Syngenta to assist in decision-making and optimise spray timing to target the second generation of aphids, based on a model using the T-sum 170 DD threshold https://www.syngenta.co.uk/agronomy-tools/BYDV-assist. A notification is given at 145DD to allow a couple of days to inspect the field and another when the 170DD threshold is reached. If aphids are present when the 170DD is imminent or occurring an application spray is recommended. The included spray application guide can be used to plan optimum application timings, once treatment is warranted.

Cultural control is an important tool for minimising BYDV risk. As aphid migrations normally cease by early November, delaying drilling can avoid the majority of aphid migration (Harrington *et al.*, 1999) and has been shown to significantly reduce aphid feeding pressure and increase yield (Royer *et al*., 2005). However, this approach may be less effective in years when mild, dry conditions allow aphid migration to continue into December. Nor may it fit with

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cultivation programmes, especially if weather conditions deteriorate in the autumn (Dewar *et al*., 2016). It is important to consider that later drilling can reduce yield potential (Spink *et al*. 2004) and a better understanding of the trade-off between delayed sowing and reduced BYDV is needed.

Reducing the presence of volunteer cereals and grass weeds (known as the 'green bridge') minimises sources of the virus and movement of vectors (especially *S. avenae*) into the newly emerging crop (Dewar *et al*., 2016). Rotation can help to minimise the build-up of BYDV sources and vector populations. For example, a field that had been in wheat and barley for four years continuously was the only site at which BYDV was found in a 2010 survey (out of 716 samples) (Flint, 2015). Cultivations can also affect risk, with BYDV infection greater following conventional-tillage than non-inversion or minimum tillage (Kendall *et al*., 1991; Kennedy *et al*., 2010; Kennedy & Connery, 2012). This is thought to be due to habitat disturbance affecting the activity of the aphids and their natural enemies (Prew *et al*., 1988; Kendall *et al*., 1991).

The importance of biological control for BYDV vectors has been recognised (Plantegenest *et al*., 2001; Ramsden *et al*., 2016), with a range of natural enemies targeting cereal aphids, including spiders, rove beetles, ladybirds, ground beetles and parasitic wasps (Holland & Oakley 2007). There is also great potential to improve the way that land surrounding fields is managed to enhance the populations of natural enemies (Olson and Wäckers, 2006). Agricultural landscapes that provide a greater diversity of resources have the capacity to support a larger, more diverse and more robust population of beneficial natural enemies (Greenop *et al*., 2018). Therefore, modifying landscape management has great potential to limit cereal aphid populations before and after migration into emerging crops. Furthermore, it has been shown that viruliferous aphids are more vulnerable to parasitism than uninfected aphids (de Oliveira *et al*., 2014). Biological control of BYDV vectors is reviewed in further detail in Dewar *et al.* (2016).

BaYMV: As for BaMMV above, the use of resistant cultivars is the main method of combatting BaYMV. Resistant cultivars are commercially available (see Breeding for aphid resistance and host resistance and/or tolerance section below) and all varieties in the 2019 RL list in the UK carry resistance (AHDB, 2019). As cool temperatures appear to slow the development of the virus, delayed drilling may lead to disease avoidance (Laing, 1989). Indeed Plumb *et al.* (1986) found lower levels of infection in crops sown in November than in October. Methods for managing *P. graminis* are as in BaMMV above.

SBCMV: Due to the difficulty controlling *P. graminis* (see BaMMV above), the use of resistant cultivars is considered the only way of combatting SBCMV (Budge *et al.*, 2008; Kühne, 2009).

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Hariri *et al.* (2001) suggest that growing mixed stands of resistant and sensitive strains can reduce disease levels. Cultural control, such as crop rotation and delayed sowing, is considered ineffective for the management of the virus (Kühne, 2009). However, it is worth that the only sample of SBCMV found in a survey of wheat viruses in 2010 (out of 716 samples) came from a field which had been in wheat for 20 years (Flint, 2015), suggesting that lack of rotation may increase risk.

WDV: As this virus is rare in the UK, no control measures currently exist.

For viruses (or virus isolates) not yet found in the UK, effective quarantine and border inspections are needed to prevent their arrival. However, Bacon *et al*. (2012) identified that border controls in Europe need improvement. Furthermore, wheat seed imported into the UK is not tested, presenting a potential means of entry for seed-borne viruses (Flint, 2015).

Chemical and cultural management options in use/under research in other countries their efficacy/efficiency and their feasibility for use here.

BaMMV: Control of the virus in other countries is similar to that in the UK, with varietal resistance the primary method of management (Kühne, 2009). In terms of *P. graminis* control, research has shown the potential for controlling the related *P. betae* using biocontrol agents such as soil saprophytic *Trichoderma* spp. (Jakubíková *et al*., 2006; Yilmaz & Tunali, 2010). This presents the possibility of investigating similar approaches for *P. graminis*, although applying such biocontrol agents on a wide-scale may be prohibitively expensive.

BYDV: As in the UK, control of the virus elsewhere in the world relies on chemical control of the vectors. Other control options are as described in the Chemical and cultural management options section above (Serfling *et al*., 2016). A number of BYDV models have been developed elsewhere, e.g. Fabre *et al.*, 2006; Thackray *et al*., 2009; Walls *et al*., 2016 and Enders *et al*., 2018 (see the Modelling section above). It is difficult to know how widely these DSS are used but it would be worthwhile investigating their applicability to UK conditions. Research has also shown the potential for essential oils in controlling BYDV vectors (Sánchez Chopa and Descamps, 2012; Bushra *et al*., 2014; Grul'ová *et al*., 2017; Shah *et al*., 2017). Such plant-based insecticides may be at lower risk of developing resistance against them as they often act on multiple sites (Tripathi *et al*., 2009; Rattan, 2010).

BaYMV: Control of the virus in other countries is similar to that in the UK, with varietal resistance the primary method of management (Kühne, 2009). See BaMMV above for potential *P. graminis* control methods.

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SBCMV: Control of the virus in other countries is similar to that in the UK, with varietal resistance the primary method of management (Serfling *et al*., 2016). See BaMMV above for potential *P. graminis* control methods.

SBWMV: The primary means of controlling the virus is sowing resistant varieties (seethe Breeding for aphid resistance and host resistance and/or tolerance section below) (Serfling *et al*., 2016). Delayed drilling has also been shown to reduce infection (Robert, 2014, Serfling *et al*., 2016).

WDV: Control of this virus remains difficult. The cultivation of resistant varieties is considered the most effective method of managing the virus but such resistance is elusive (the Breeding for aphid resistance and host resistance and/or tolerance section below). Chemical control of the leafhopper vector is not considered effective due to the high mobility of the insects (Serfling *et al*., 2016). Late sowing can reduce virus pressure (Lindblad & Arenö, 2002). Plants become resistant after GS31 meaning that control of the virus after this stage is unnecessary (Lindblad & Sigvald 2004). The proportion of the vector carrying the virus was found to range from 0 to 79% depending on the location and year (Manurung *et al*., 2004). Diagnostic methods able to provide 'real time' information on the size of viruliferous leafhopper populations, coupled with predictive models, may help understand risk and improve control programmes.

WSMV: Chemical control of the vector, *A*. *tosichella*, is not considered economical with currently available acaricides (Serfling *et al*., 2016). The poor performance of acaricides is likely due to the difficulty in targeting the vector, which spends much of its time under leaf sheaths or on curled leaves on the whorl (Skoracka *et al*., 2018). Cultural control is an important tool (Serfling *et al*., 2016), with both delayed drilling (Wosula *et al.*, 2018) and the removal of volunteers and weed hosts (Ranabhat *et al.*, 2018) reducing virus risk. DSS tools have also been developed (seethe Modelling section above).

WSSMV: Due to the difficulty in controlling the vector, *P. graminis*, management of the virus relies largely on the use of resistant varieties (Kühne, 2009) (seethe Breeding for aphid resistance and host resistance and/or tolerance section below). Delayed drilling has also been shown to reduce infection (Robert, 2014). See BaMMV above for potential *P. graminis* control methods.

WYMV: As for WSSMV, virus control relies on the use of resistant varieties (see the Breeding for aphid resistance and host resistance and/or tolerance section below) as management of the vector is difficult (Kühne, 2009). See BaMMV above for potential *P. graminis* control methods.

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For viruses generally, it has been suggested that antiviral drugs could be used as control agents (Borodavka *et al*., 2012) and that these could be applied as nanoparticle additives to fertiliser applications (Flint, 2014).

Breeding for aphid resistance and host resistance and/or tolerance

BaMMV: Studies have shown that resistance to the virus is relatively frequent in the primary barley gene pool (Ordon *et al.*, 2009). The recessive resistance gene, rym4, was the only known source of resistance for almost twenty years and has been bred into the majority of resistant commercial barley varieties (Kühne, 2009). In the UK, all varieties in the 2019 RL are resistant to BaMMV (AHDB, 2019). Over the last forty years, work has identified multiple resistance sources in barley (Kühne, 2009; Ordon *et al.*, 2009; Silvar *et al*., 2010; Yang *et al*., 2013; Perovic *et al*., 2014; Yang *et al*., 2014; Johnstone *et al*., 2015). Yang *et al*. (2017) found that barley from East Asia carries the highest number of resistance genes, likely because the virus originated from the area. These resistance sources represent potential sources of resistance for global breeding programmes. An alternative to viral resistance would be breeding for resistance to the vector, *P. graminis*, however there has been little success in this regard, although a form of resistance has been detected in *H. bulbosum* (Kühne, 2009).

BYDV: No true resistance has been identified in wheat (Jaroseva *et al.*, 2016). The *Bdv1* gene confers tolerance to BYDV-MAV and originates from a Brazilian wheat cultivar (van Ginkel & Henry, 2002). High levels of resistance have been identified in several Triticeae species (Barloy *et al.*, 2003) and much effort has been made toward breeding this into wheat (see Jaroseva *et al.*, 2016).

Four genes associated with resistance/tolerance to BYDV have been identified in barley; *Ryd1*, *Ryd*2, *Ryd*3 and *Ryd4Hb* (Jaroseva *et al.*, 2016). *Ryd1* confers intermediate tolerance and is rarely used in breeding programmes due to its low efficiency (Jaroseva *et al.*, 2016). *Ryd*2 is the main source of resistance used in barley breeding programmes, having been introduced into many cultivars (Kosová *et al.*, 2008), and confers field tolerance to the MAV, PAV, SGV and some RPV isolates of the virus (Scholz *et al.*, 2009; Jaroseva *et al.*, 2016). It is of Ethiopian barley origin (Schaller *et al.*, 1964) and is thought to act by reducing by phloem viral replication (King *et al*., 2002). *Ryd*3 is also of Ethiopian barley origin (Niks *et al*., 2004), conferring high levels of tolerance (Jaroseva *et al.*, 2016) and resistance when combined with *Ryd*2 (Jaroseva *et al.*, 2016). Commercially available cultivars containing *Ryd*3 alone and both *Ryd*2 and *Ryd*3 have been bred (see Jaroseva *et al.*, 2016). *Ryd4Hb* is derived from *H. bulbosum* and, while it confers resistance, it is thought to be vector-based rather than true "virus resistance" (Jaroseva *et al.*, 2016). It has been transferred to barley but has not been

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incorporated into breeding programmes due to linkage drag (del Blanco *et al*., 2014). A number of QTLs providing BYDV tolerance have also been identified (see Jaroseva *et al.*, 2016). Breeding for virus resistance or tolerance in wheat and barley is reviewed in more detail in Jaroseva *et al*. (2016).

As an alternative to breeding resistance to BYDV, it may be possible to breed resistance to the vectors of the virus. In general, aphid resistance appears to be polygenic although there are examples of single dominant R-genes (Dedryver *et al.*, 2010; Dogimont *et al*., 2010). Whilst effective, R-gene mediated resistance is often highly specific to a particular genotype/biotype and can be broken down in as little as two years after commercial release in the field (McDonald and Linde, 2002). However, these R genes can be stacked to make it harder for pests to evolve counter-resistance and to provide multiple resistances to different attackers. So far breeding for such resistance to cereal aphids has concentrated on *Schizaphis graminum* and *Diuraphis noxia*, neither of which are serious pests in northern Europe (Dewar *et al*., 2016). For UK vectors of the virus, there has been little success in breeding resistance (Dedryver *et al*., 2010). Klueken *et al*. (2008) identified a wheat cultivar, 'Hybnos I' that significantly reduced the fecundity of *S. avenae* and *M. dirhodum* offspring at the seedling stage but not later stages. Girvin *et al.* (2017) identified currently available (in the USA) wheat cultivars that supressed aphid populations and were able to tolerate aphid feeding. Luo *et al.* (2019) characterised the tolerance of the winter wheat line XN98-10-35 to *S. avenae* and *R. paid* under field conditions. Barley lines based on a cross between barley cv. Lina and a wild barley accession have shown resistance to *R. padi* (Mehrabi *et al.*, 2016).

Resistance has also been identified in hosts closely related to wheat and barley. Schliephake *et al.* (2013) identified a *H. bulbosum* clone to which *R. padi* and *S. avenae* were unable to transmit BYDV, likely due to the reduced phloem feeding period being too short for successful virus infection, and is the source of *Ryd4Hb* barley resistance gene (see above). A resistant line has been identified in *Triticum monococcum* that is able to hinder virus transmission (Tanguy & Dedryver *et al.*, 2009) and drastically reduce the fecundity in *S. avenae* (Caillaud *et al.*, 1994). However, it had little effect on other vectors and the genes have proven difficult to breed into hexaploid wheat (Dedryver *et al*., 2010). Triticale lines that reduce *R. padi* developmental rate and fecundity have been identified and early attempts to cross the resistance into wheat have shown promise (Hesler *et al.*, 2007).

Genetic modification of cereals also presents some opportunities. Bruce *et al.* (2015) transformed wheat to produce aphid alarm pheromones, and while these repelled cereals aphids in laboratory trials, no reductions in aphid numbers were observed in field trials. Duan *et al.* (2018) transformed wheat to produce mannose binding lectin, a product toxic to sap-sucking insects. The transformed lines lowered aphid growth rates and increased inhibition

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rates in bioassays, and reduced aphid infestation in field trials. Hou *et al.* (2019) transformed wheat to produce dsRNA that inhibited expression of Gqa genes in *S. avenae* resulting in significantly lower reproduction levels and moulting. Breeding for aphid resistance and tolerance in cereals is reviewed in more detail in Jarošová *et al.* (2016) and Dewar *et al.* (2016). Understanding the interaction between varieties that are resistant to aphids and the impact on virus transmission is important. For instance, a wheat variety that significantly reduced aphid numbers and development also resulted in the higher production of alates (winged aphids) and, in turn, greater BYDV infection (Liu *et al.*, 2014). In general, cultivars able to slow development and reduce reproduction without affecting alate production may delay and reduce secondary spread of the virus within crops and reduce the need for insecticide applications.

BaYMV: As for the related BaMMV, resistance to BaYMV is relatively frequent in the primary barley gene pool (Ordon *et al.*, 2009). The first commercially available varieties resistant to BaYMV were developed in Japan and contained *rym5* (Kühne, 2009), however this was soon overcome by a new pathotype (Kashiwazaki *et al.*, 1989). The first virus resistance varieties in Europe contained the *rym4* resistance gene and this remained the source of resistance for around twenty years (Kühne, 2009). However, this resistance was overcome by a new pathotype in the 1980s ((Kühne, 2009). Multiple new sources of resistance have been identified in the last forty years (Kühne, 2009; Ordon *et al.*, 2009; Sedláček *et al*., 2010; Hofinger *et al*., 2011; Kai *et al.*, 2012; Yang *et al*., 2013; You & Shirako, 2013; Perovic *et al*., 2014; Yang *et al*., 2014). In the UK, all varieties in the 2019 RL are resistant to BaYMV (AHDB, 2019). As the virus originates in East Asia, barley from this region carries the highest number of resistance genes, presenting potential resistance sources for breeding programmes (Yang *et al*., 2017). See BaMMV above for efforts to breed resistance to *P. graminis.*

SBCMV: Resistance has been identified in *T. aestivum*, *T. durum*, *T. monococcum* and *Thinopyrum intermedium* (Serfling *et al*., 2016). Resistance to SBCMV is based on the inhibition of virus movement from the roots to the stem (Ordon *et al.*, 2009). Two major resistance genes, *Sbm1* and *Sbm2*, have been identified in wheat (Bass *et al*., 2006; Bayles *et al.*, 2007) and some breeders are incorporating these into their breeding programmes (Flint, 2015). Budge *et al.* (2008b) identified several cultivars exhibiting resistance, including Charger, Claire and Hereward. Lines carrying both *Sbm1* and *Sbm2* had significantly lower levels of virus than lines can carrying either of the genes, suggesting that the resistance is complementary (Bayles *et al.*, 2007). It is likely that cultivars resistant to SBCMV will also exhibit resistance to the closely related SBWMV (Bayles *et al.*, 2007). Resistance is reviewed in greater detail in Kühne (2009) and Ordon *et al.* (2009). Further research into the

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characterisation and breeding of resistance includes Perovic *et al*. (2009), Maccaferri *et al*. (2011) and Russo *et al.* (2012). See BaMMV above for efforts to breed resistance to *P. graminis.*

SBWMV: Many wheat cultivars with resistance to SBWMV are available in the USA, Japan and Brazil (Kühne, 2009; Zhang *et al*., 2017; Mason *et al*., 2018). The resistance is derived from a single dominant resistance gene (Ordon *et al.*, 2009). Recent work has identified further resistance genes and QTLs (Zhang *et al*, 2011; Hao *et al*., 2012; Liu *et al*., 2014). Further resistance sources have been identified in related plants such as the wild wheat species *Aegilops tauschii* (Hall *et al*., 2009; Zhang *et al*, 2011). Work also suggests that temperature affects the expression of resistance (Drumm Meyers *et al*., 1993). It is thought likely that cultivars resistant to SBCMV will also exhibit resistance to the closely related SBCMV (Bayles *et al.*, 2007). See BaMMV above for efforts to breed resistance to *P. graminis.*

WDV: Few natural sources of resistance to WDV have been identified. Ordon *et al.*'s (2009) review found that despite extensive screening efforts only a few wheat genotypes with partial resistance or tolerance and just one winter barley cultivar with tolerance have been found. Since this review Benkovics *et al.* (2010) identified further partial resistance in wheat. Work has also identified *A. tauschii* as a potential source of resistance (Nygren *et al*., 2015). GM approaches show more promise in developing host resistance. Kis *et al.* (2016) transformed barley to exhibit miRNA-mediated resistance that was effective at low temperatures (a prerequisite given the conditions during at infection). Cejnar *et al.* (2018) developed transgenic barley in which infection was delayed by approx. two weeks. Recent work has demonstrated the potential for CRISPR methods to develop WDV resistance in barley (Kis *et al*., 2019).

WSMV: Resistance to the virus was first detected in perennial Triticeae relatives, resulting in the identification of three resistance genes; *Wsm1*, *Wsm2* and *Wsm3* (Singh *et al*., 2018). *Wsm1* and *Wsm2* have been incorporated into commercially available cultivars, although these are not available in Europe (Singh *et al*., 2018). Additionally, the resistance that these genes confer are temperature sensitive and can be linked to agronomically negative traits (Cruz *et al*., 2014). Furthermore, resistance breaking isolates to *Wsm2* have been detected recently in the USA (Kumssa *et al*., 2019). *Wsm3* is less temperature sensitive but has not yet been bred into commercial varieties (Singh *et al*., 2018). The lack of natural resistance sources in wheat has increased the importance of alternative sources, with potential resistance identified in wheat relatives (Wanlong *et al*., 2019). Transgenic wheat with resistance based on RNA interference has been developed, resulting in stable immunity to WSMV (Fahim *et al*., 2010; Fahim *et al*., 2012; Cruz *et al*., 2014).

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Resistance to the vector, *A*. *tosichella*, has been identified in wheat and several other grass species, including hybrids between spring wheat and *Agropyron glaucum* (Aguirre-Rojas *et al*., 2017; Dhakal *et al*., 2018 Singh *et al*., 2018). Recent work has successfully developed wheat lines with resistance to both WSMV and *A*. *tosichella* (Chuang *et al*., 2017). While no effective resistance against *A*. *tosichella* is available in commercial wheat cultivars, such an approach presents good potential to reduce losses from the pest and virus (Singh *et al*., 2018). Resistance to the virus and the vector is reviewed in greater detail in Ordon *et al.* (2009) and Singh *et al*. (2018).

WSSMV: Resistance has been identified in several wheat cultivars and experimental lines (Serfling *et al*., 2016) and there are many commercially available resistant cultivars (Kühne, 2009). The majority of European bread varieties are thought to be resistant (Hourcade *et al*., 2019), indeed Budge *et al.* (2008b) found that all the UK cultivars they tested were resistant to WSSMV. There may also be a link between SBCMV and WSSMV resistance, with several UK and French SBCMV resistant varieties also exhibiting resistance to WSSMV (Budge *et al.*, 2008; Kühne, 2009). Resistance is reported to be controlled by up to three major genes and several QTLs (Khan *et al*., 2000; Kühne, 2009; Holtz *et al*., 2017) but the genetic basis of the current resistance is narrow and further sources are needed (Kühne, 2009). To this end, resistance has also been detected in other grasses, including rye (Li *et al.*, 2007; Erath *et al* 2016) and *Haynaldia villosa* (Zhang *et al*., 2005). Genetic modification of wheat has also successfully resulted in reduced susceptibility to the virus (Kühne, 2009). See BaMMV above for efforts to breed resistance to *P. graminis.* Resistance to WSSMV is reviewed in greater detail in Kühne (2009), Ordon *et al.* (2009) and Singh *et al*. (2018).

WYMV: Sources of resistance have been identified in Japanese, Chinese and European varieties and numerous resistant varieties are available to growers (Kühne, 2009; Serfling *et al*., 2016). The resistance has been mapped in some varieties and several major genes and QTLs are thought to be involved (Kühne, 2009; Serfling *et al*., 2016; Xiao *et al*., 2016). As an alternative to traditional breeding approaches, transgenic lines have been produced that range from being less susceptible to viral infection to having high and durable resistance (Kühne, 2009; Chen *et al*., 2014).

Genetic modification and CRISPR technology

As described in the Modelling section (above), ELISA can detect BYDV in leaf samples and single aphids but is not sufficiently sensitive and can lead to false negatives when measuring low levels of viral titre in viruliferous aphids (Canning *et al.*, 1999). Recent advances in diagnostic methods (Chomič *et al.*, 2010; Foster & Williamson, 2015) present the opportunity for rapid testing of aphids caught in traps to provide ‘real time’ data on the proportion that are

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viruliferous. However, insect mRNA and viral RNA are unstable and although solvents can be added to water traps to improve stability, these evaporate quickly (Flint, 2014). Therefore, assessing viral levels using this method currently requires water traps to be monitored frequently. Several successful attempts at genetically modifying wheat and barley to create transgenic varieties resistant to BYDV or its vectors have occurred (e.g. McGrath *et al*., 1997; Wang *et al*., 2000; Yan *et al*., 2006; Bruce *et al*., 2015), and are fully reviewed in Jarošová *et al*. (2016).

The genetic modification of wheat with genes from BaYMV has been demonstrated (Karunaratne *et al.*, 1996). Transgenic plants containing WDV genes have improved resistance to the virus (Kis *et al*., 2016; Cejnar *et al*., 2018). Genetic modification of wheat by the introduction of WSMV genes has resulted in high levels of resistance to the virus (Fahim *et al*., 2010; Fahim *et al*., 2012; Cruz *et al*., 2014). Genetic modification of wheat with genes from WYMV has resulted in resistance to the virus (Dong *et al*., 2002; Chen *et al*., 2014).

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Virus management in oilseed rape

Oilseed rape (OSR) (*Brassica napus* L.) is a highly important break crop in the UK. At its peak, OSR was sown across 756,000 ha in 2012 but the area has since reduced to 601,000 ha in 2018 (Defra, 2019). The reduction in cultivation has been identified as being largely due to two factors; price fluctuation and poor pest control (Dewar & Walters, 2016). The price of OSR has fluctuated in recent years, dropping to £250-270 per tonne in 2016, in part due to competition from other oil-producing crops such as palm and soy-bean (Dewar & Walters, 2016). The loss of neonicotinoid seed treatments and the increasing issue of insecticide resistance has reduced pest control options and substantially increased the risk of yield reductions and crop failure. However, markets are limited for alternative break crops (Dewar & Walters, 2016) meaning that OSR remains the preferable break crop for many growers.

The impact of viruses on OSR is of major concern to the industry, especially with reductions in the availability of chemical control options in recent years. There are several OSR-infecting viruses in the UK with *turnip yellows virus* (TuYV) having the greatest impact in terms of the proportion of crops affected and yield reductions. On-farm OSR yields have barely increased since 1985, with the long-term average being 3.1 t/ha in comparison to an estimated yield potential of greater than 6.5 t/ha (AHDB, 2018). It has been suggested that TuYV is a primary reason for OSR not reaching its full genetic yield potential in the UK (Stevens *et al.*, 2008).

Current viruses present in the UK

There are four potential viruses of OSR; TuYV, *cauliflower mosaic virus* (CaMV), *turnip mosaic virus* (TuMV) and *turnip yellow mosaic virus* (TYMV, Table 10). Of these TuYV is by far the most important in the UK. It causes stunting, reduced leaf area and fewer primary branches in OSR, with the potential to cause yield reductions of between 23-30% in the UK (Stevens *et al*, 2008; HGCA, 2012). It is estimated that the virus affects 60% of OSR crops nationally (Clarke *et al*., 2009) but the proportion varies annually and regionally, with up to 80% of crops infected in some areas of the UK in 2015 (Lüders, 2017). In general, crops at highest risk are in Southern England and other areas with intensive brassica cultivation (Walsh, 2014). Nicholls (2013) calculated that based on an average yield loss of 15%, annual yield reductions in unprotected crops would be 206,010 t or £67 million.

Hardwick *et al*. (1994), reported on the incidence of virus diseases in OSR in 1991/92 and 1992/93. A total of 62% of crops contained TuYV (reported as BWYV but since reclassified as TuYV) in 1991/92 with an average of 28% plants infected, and 42% of crops in 1992/93 contained the virus with an average of 12% plants infected. The respective figures for CaMV and TuMV were 14% and 3% of crops containing each virus in 1991/92 with 5% and <1%

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plants infected, and 25% and 14% of crops containing each virus in 1992/93 with an average of 7% and 5% plants infected.

Table 10. Summary of viruses reported on oilseed rape in the United Kingdom, together with, where available, information on their mode of transmission and their vector. Viruses highlighted in bold italics are further discussed in the present Section.

Virus	Acronym	Genus	Mode of transmission	Vector
Turnip yellows virus	***TuYV***	***Polerovirus***	***Persistent; aphid transmitted***	***Aphis craccivora, A. gossypii, Acyrthosiphon solani, Brachycaudus helichrysi, Brevicoryne brassicae, Macrosiphum euphorbiae, Myzus ornatus, M. persicae***
Cauliflower mosaic virus	CaMV	Caulimovirus	Semi persistent; aphid transmitted	*B. brassicae, M. persicae* and at least 25 other aphid species
Turnip mosaic virus	TuMV	Potyvirus	Non persistent; aphid transmitted	Transmitted by 40-50 aphid spp*. especially M. persicae and B. brassicae*
Turnip yellow mosaic virus	TYMV	Tymovirus	Biting insects, including beetles and the mustard beetle	Phyllotreta and Psylloides species (flea beetles) and *Phaedon cochleariae* (the mustard beetle) in Europe, Pedilophorus spp. in Australia

TuYV, CaMV and TuMV are all transmitted by aphids of which the peach-potato aphid (*M. persicae*) is by far the most important species. TuYV is transmitted in a persistent manner whereas both CaMV and TuMV are transmitted non-persistently. TYMV is transmitted by biting insects such as weevils and flea beetles, including cabbage stem weevil and cabbage stem flea beetle.There is however, very little additional information on CaMV, TuMV or TYMV in the UK so the remainder of this section will concentrate on TuYV. Stevens *et al*., (2008) provide an extensive review of TuYV in OSR and much of the information in this section is taken from that source.

New emerging viruses

There are currently no new emerging viruses of OSR.

Virus vectors and alternative hosts

Vectors of key viruses

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TuYV is potentially transmitted by a wide range of aphid species (Schliephake *et al*., 2000, Table 11) but in practice it is rare to find anything other than peach-potato aphid and mealy cabbage aphid in OSR. Of these peach-potato aphid is by far the most important vector (Stevens *et al*., 1995). The virus is transmitted in a persistent (circulative, non-propagative) manner. This means that once it has been acquired by the aphid it retains the ability to transmit it even after moulting, although the virus does not pass through to the progeny (Schliephake *et al*., 2000; Stevens *et al*., 2006). TuYV is not thought to be mechanically or seed transmissible as the virus is confined to the vascular tissue and virus particles are unable to enter the ovule as this has no vascular connectivity with the parent plant.

Table 11. Insect vectors of viruses of oilseed rape (the main vector of TuYV is highlighted)

Virus	**Aphid vector**	
	Latin name	**Common name**
Turnip yellows virus	*Acyrthosiphon pisum*	Pea aphid
	Aphis gossypii	Melon-cotton aphid
	Aulacorthum circumflexum	Lily aphid
	Aulacorthum solani	Foxglove aphid
	Brachycorynella asparagi	Asparagus aphid
	Brevicoryne brassicae	Mealy cabbage aphid
	Cavariella aegopodii	Willow-carrot aphid
	Macrosiphoniella sanborni	Chrysanthemum aphid
	Macrosiphum albifrons	Lupin aphid
	Macrosiphum euphorbiae	Potato aphid
	Myzus nicotianae	Tobacco aphid
	Myzus persicae	**Peach-potato aphid**
	Nasonovia ribisnigri	Currant-lettuce aphid
	Pentatrichopus fragaefolii	Strawberry aphid
	Rhopalosiphum maidis	Cereal leaf aphid
	Rhopalosiphum padi	Bird cherry-oat aphid
	Sitobion avenae	Grain aphid

As TuYV is phloem limited, aphids need to ingest infected sap to acquire the virus particles. For successful transmission to occur the virus particles then have to pass through the gut wall and the accessory salivary gland membrane and eventually accumulate in the accessory salivary gland. The particles are then injected into the plant during penetration of the aphid's stylet during feeding.

The virus acquisition period can be as short as 15 minutes but the latent period (the time taken between an aphid acquiring the virus before being able to transmit the particles to a new host) is usually at least 24 hours and can be as long as four days. Once an aphid is able

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to transmit the virus to a new host the inoculation access period can be as short as 10-30 minutes but the efficiency of transmission increases the longer the aphid feeds on the plant.

The peach-potato aphid is a highly efficient vector of TuYV with transmission rates of over 90% having been reported experimentally (Schliephake *et al*., 2000). The proportion of viruliferous aphids (those carrying TuYV) varies regionally and annually from <5% to 72% (Stevens *et al*., 2008) but can be as high as 93% (Katschnig *et al*. 2017). The variation in the number of viruliferous aphids depends largely on weather conditions, which influence the survival and fecundity of aphid populations and the number of available sources of the virus.

Other aphid species that play a more limited role in the transmission of TuYV are potato aphid (*Macrosiphum euphorbiae*) and mealy cabbage aphid (*Brevicoryne brassicae*). However, these species have much lower rates of virus transmission (Schliephake *et al*., 2000), and fewer individuals tend to carry the virus (Stevens *et al*., 1995). Herrbach (1994) showed that French clones of mealy cabbage aphid were unable to transmit the virus.

Alternate host species of key viruses

TuYV is primarily of interest as a pathogen of OSR but it can also infect a wide range of both crop and weed species (Tables 12 & 13). The diverse range of plant species susceptible to TuYV increases the potential reservoir of hosts in which the virus can survive throughout the winter, and provides a source for future virus outbreaks (Smith and Hinckes, 1985; Stevens *et al*., 1994; Latham *et al*., 2003).

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Table 12. Crop species that can act as alternative hosts for TuYV

Latin name	Common name
Brassicaceae	
Brassica juncea	Indian mustard
Brassica napus ssp. *napus*	Oilseed rape
Brassica napus ssp. *rapifera*	Swede
Brassica oleracea var *acephala*	Kale
Brassica oleracea var *alboglabra*	Chinese kale
Brassica oleracea var *botrytis*	Cauliflower
Brassica oleracea var *capitata*	Cabbage
Brassica oleracea var *capitata*	Cabbage
Brassica oleracea var *gemmifera*	Brussels sprouts
Brassica oleracea var *gongylodes*	Kohlrabi
Brassica oleracea var *italica*	Calabrese
Brassica oleracea var *sabauda*	Savoy cabbage
Brassica rapa ssp. *chinensis*	Pak choi
Brassica rapa ssp. *narinosa*	Broadbeaked mustard
Brassica rapa ssp.*oleifera*	Turnip rape
Brassica rapa ssp.	False pak choi
Brassica rapa ssp. *pekinensis*	Chinese cabbage
Brassica rapa ssp. *perviridis*	Spinach mustard
Brassica rapa ssp. *rapifera*	Turnip
Lepidium sativum	Garden cress
Raphanus sativus var. *niger*	Winter radish
Raphanus sativus var. *oleiformis*	Fodder radish
Sinapis alba	White mustard
Chenopodiaceae	
Spinacea oleracea	Spinach
Compositae	
Lactuca sativa	Lettuce
Fabaceae	
Cicer arietinum	Chickpea
Lupinus albus	White lupin
Pisum sativum	Pea
Vicia faba	Broad bean

Table 13. Weed species that can act as an alternative host for TuYV

Latin name	Common name	Latin name	Common name
Asteraceae		Fumarianceae	

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Latin name	Common name	Latin name	Common name
Conzya spp.	Fleabane	*Fumaria officinalis*	Common Fumitory
Matricaria perforata	Scentless mayweed	Hydrophyllaceae	
Brassicacae		*Phacelia tanacetifolia*	Scorpion weed
Arabidopsis thaliana	Thale cress	Lamiaceae	
Brassica carinata	Abyssinian cabbage	*Lamium amplexicaule*	Henbit deadnettle
Brassica nigra	Black mustard	*Lamium purpureum*	Purple deadnettle
Brassica rapa ssp. *rapa*	Field mustard	Papaveraceae	
Brassica rapa ssp. *sylvestris*	Wild turnip	*Papaver rhoeas*	Corn poppy
Capsella bursapastoris	Shepherd's purse	Polemoniaceae	
Camelina sativa	Gold-of-pleasure	*Navarretia squarrosa*	Stinkweed
Lepidium campestre	Field pepperweed	Portulaceae	
Lepidium ruderale	Roadside pepperweed	*Montia perfoliata*	Miner's lettuce
Raphanus raphanistrum	Wild radish	Primulaceae	
R. sativus var. *albus*		*Anagallis arvensis*	Scarlet pimpernel
R. sativus var. *violaceus*	White radish	Scrophulariaceae	
Sinapis arvensis	Wild mustard	*Veronica arvensis*	Corn speedwell
Thlaspi arvense	Fanweed	*Veronica persica*	Common field speedwell
Caryophyllaceae		Solanacecae	
Stellaria media	Common chickweed	*N. benthamiana*	
Spergula arvensis	Corn spurry	*Nicotiana clevelandii*	Cleveland's tobacco
Compositae		*Nicotiana occidentalis*	
Chrysanthemum segetum	Corn marigold	*Physalis floridiana*	
Senecio vulgaris	Groundsel	*Physalis pubescens*	Hairy nightshade
Taraxacum officinale	Common dandelion	*Solanum nigrum*	Blackberry nightshade
Zinnia peruviana	Peruvian zinnia	Urticaceae	
Cucurbitaceae		*Urtica urens*	Annual nettle
Citrullus lanatus	Afghan (wild) melon	Valerianaceae	
Fabaceae		*Valerianella locusta*	Lewiston cornsalad
Lupinus luteus	Yellow lupin	Violaceae	
Ornithopus sativus	Pink serradella	*Viola arvensis*	Field pansy
Trifolium resupinatum	Persian clover		

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Early detection, surveillance, and management of viruses

As infection with TuYV is often asymptomatic, diagnostic methods to confirm the presence and concentration of the viral infection are crucial. The techniques available for detection and identification of TuYV are listed in Table 14.

Table 14. Methods for detection and identification of TuYV

Relevant virus	Detection method
TuYV	Transmission tests, ELISA, Western blot Immunosorbent electron microscopy, PCR, Riboprobes, RFLP, RT-PCR, LAMP

Detection and Identification Methods Based on Biological Properties

Oilseed rape infected with TuYV produces a wide range of symptoms. These often go unnoticed as they resemble those of stress and nutrient-deficiency. This includes reddening of the leaf margins and interveinal yellowing and reddening. Infected plants tend to show red or purple discolouration initially on older leaves, but symptoms can extend to all leaves by early summer.

TuYV infection in weed species can also be symptomless. Many hosts can show interveinal yellowing or reddening which may also be accompanied by dwarfing. Some weed species develop distinctive symptoms when infected. For example, the older leaves of shepherd's purse (*Capsella bursa-pastoris*) become yellow, curled and brittle when infected, whilst *Montia perfoliata* (Miner's lettuce) turns red. Spinach leaves develop mild yellowing discolouration in interveinal areas and at the leaf tip whilst lettuce shows chlorotic blotching which later develops into severe interveinal yellowing. Also, symptoms typical of TuYV can be wrongly diagnosed as nutrient deficiency, water stress, frost damage or even natural senescence. In England, between 1968 and 1970, many lettuce crops were thought to be suffering from magnesium deficiency, when actually they were infected with TuYV.

Polioviruses are spherical, non-enveloped particles approximately 25-30 nm in diameter (Coleman, 2013). The protein shell is composed of 180 coat proteins, orientated into T=3 icosahedral symmetry. Virus particles contain a single-stranded positive-sense RNA molecule, typically of about 6 kilobasepair (kbp) (Hull, 2001). This RNA is infectious and serves as both the genome and viral messenger RNA. Differentiation between TuYV and the closely related beet western yellows virus can be achieved through transmission tests as the former is non-pathogenic in sugar beet (*Beta vulgaris*) (Graichen & Rabenstein, 1996). Characterisation of TuYV has been developed using ELISA (D'Arcy *et al*., 1989; Smith *et al*.,

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1996), Western blot (Fomitcheva *et al.*, 2004) and immunosorbent electron microscopy (Hipper *et al*., 2014).

Detection and Identification Methods Based on Viral Nucleic Acid

Detection of TuYV has been developed using PCR (Jones *et al*., 1991), riboprobes (Lemaire *et al*., 1995) and RT-PCR (Hauser *et al*., 2000). RFLP can also be used for identification but interpretation of results can be difficult if point mutations alter the RFLP patterns (Hauser *et al*., 2000). Foster and Williamson (2015) developed a rapid PCR-based diagnostic assay for TuYV that can be run alongside insecticide resistance assays that provide a 'real-time' picture of both resistance status and the virus transmission potential of peach-potato aphid populations collected from OSR. (Congdon, Kehoe *et al*. 2019) recently developed a LAMP diagnostic assay able to detect TuYV (Australian isolates) in leaf material and its vector, *M. persicae*. This method is quicker and cheaper than alternative diagnostic techniques. It is also able to detect the virus in *M. persicae* stored for eight weeks in various trapping and storage substrates.

Novel technologies

No literature was found on other novel technologies for improved detection of TuYV.

Modelling

A model to simulate yield losses due to virus in OSR was found to successfully predict virus spread at 83% of sites across 3 years in Australia (Maling *et al*., 2010). Important risk factors identified included the availability of non-crop viral inoculum, the amount of food available for vectors and the timing of vector arrival into the crop. Development of models that provide advice to growers in the UK on optimal spray timings to minimise virus transmission would be very useful, especially given the limited number of sprays currently allowed in the autumn With the recent developments in detection technology allowing the number of viruliferous peach-potato aphids to be determined quickly and cheaply this provides the potential for data on the proportion of viruliferous aphids to significantly improve the accuracy of decision support systems (DSS).

Chemical and cultural management options

Chemical control of the aphid vector is most commonly used means of combatting TuYV. However, as the main vector, the peach-potato aphid, is resistant to organophosphate, carbamate and pyrethroid insecticides (Anon, 2018a) the majority of sprays are likely to be neonicotinoids (thiacloprid, acetamiprid) or alternative modes of action such as flonicamid (chordontal organ modulator) and pymetrozine (pyridine azomethine derivative). No

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resistance has been detected in peach-potato aphids to neonicotinoids, pyridine azomethine derivatives or chordontal organ modulators in the UK. There are restrictions on the number of sprays permitted for non-pyrethroid sprays (Table 15). While pyrethroid sprays are available they are highly likely to be ineffective due to resistance and are more likely to harm beneficial insects in the crop. If an insecticide treatment is deemed necessary, products should be applied at their full label rate. Applying insecticides below label rates can lead to a subsequent increase in resistance problems (Anon, 2018b).

The general advice on control of TuYV is to monitor pests, particularly during the period in which the crop is vulnerable and during the early stages of infestation. Monitoring should continue for as long as conditions remain conducive for aphid migration (Anon, 2018b). For example, in the autumn aphids will fly if temperatures are above 15°C but continue to move within crops at relatively low temperatures. It is hard to be precise about the level of frost needed to deliver a knock-out blow but three to five consecutive days with grass minima dropping below -6°C should cause high mortality. Monitoring tools can be used to help time crop inspections for aphids such as those available from the Rothamsted Insect Survey and AHDB Aphid News.

Table 15. Aphicides available in oilseed rape in the UK (as at May 2019), along with the mode of action (MoA), restrictions on use and notes on current UK resistance (based on Anon, 2018). † Where there is 'no limit' specified for the maximum permitted number of applications, the dose is expressed as a maximum individual and maximum total dose. †† One application permitted in autumn for peach–potato aphid control, with an additional application permitted in spring for pollen beetle control (neonicotinoid restrictions limit total number of any neonicotinoid containing product to two applications on OSR).

Mode of action (chemical group)	**Active ingredient(s)**	**Maximum permitted number of applications†**	**Peach-potato resistance in the UK**
3a (Pyrethroids)	Deltamethrin	No limit	Strong resistance widespread
3a (Pyrethroids)	Lamda-cyhalothrin	No limit	Strong resistance widespread
3a (Pyrethroids)	Tau-fluvalinate	No limit	Strong resistance widespread
4a (Neonicotinoids)	Acetamiprid	1	No resistance
4a (Neonicotinoids)	Thiacloprid	1††	No resistance

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9B (Pyridine azomethine derivatives)	Pymetrozine	1 up to GS59	No resistance
29 (Chordotonal organ modulators)	Flonicamid	1 up to GS18	No resistance

As only a single spray application is approved of each product to which the vector is not resistant (Table 15) the timing of treatment is critical. Stevens and Clark (2010) showed that in plots sequentially inoculated with aphids carrying TuYV from September through to March greatest yield losses were observed in plots from the earliest infections. This suggests that an insecticide should be applied as soon as aphids are seen in the crop. However, this will not protect against re-invasion by the aphid vectors if their migration is prolonged. An alternative approach is to delay sprays where aphid numbers are low until monitoring tools indicate that no further aphids are being caught. In the UK this is likely to be about mid-November. Although this approach may result in some virus infection it should not have a significant impact on yield if pest numbers have been low. Where aphids can be easily found in crops, an earlier spray may be advisable. The relative efficacy of these contrasting approaches has not been tested experimentally. Nevertheless, secondary spread of TuYV within the crop, following the initial infection event, is also thought to have an important effect on infection rate (Stevens *et al*, 2008). Work has shown that where just 5% of a crop is infected in October, infection rates the following spring were as high as 50% (Smith & Hinckes, 1985). It is thought that the main determinants of secondary spread are aphid movement and population size (Stevens *et al*, 2008), however little is known about the rate of spread and the factors affecting this. Control of BYDV in wheat has focused on minimising spread of the virus within the crop by using DSS to time sprays to target the second wingless generation of the aphid vectors. A similar approach may be effective in control of TuYV.

Other approaches for aphid control include the use of biopesticides or biological control using predatory insects (e.g. ladybirds, parasitic wasps) or fungal/bacterial pathogens of aphids (Bhatia *et al*., 2011). Biopesticides are becoming more common but as yet they are rarely used in arable broad acre crops. A wide range of natural predators of aphids exist which can be naturally encouraged using attractants, or artificially introduced to provide aphid biological control in crops although this option is rarely used in practice.

Recent work has investigated the potential for RNA interference (RNAi) control methods. RNAi is a natural, cellular process used by animals, plants and fungi as a means of post-transcriptional gene regulation to maintain normal growth and development, as well as a method for defence against viruses or transposable elements (Hannon, 2002). Plant-

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mediated RNA interference (PMRi) has the potential to be a good approach to TuYV control (Coleman, 2014). Mulot *et al*. (2018) used PMRi so that *M. persicae* acquire dsRNA molecules targeting *Eph* (a membrane ephrin receptor that potentially acts as a TuYV receptor), which resulted in the aphid being less able to transmit TuYV. This highly species-specific control method did not affect aphid survival and fecundity and so is likely to be an ecologically safe method of minimising TuYV impacts. However, as PMRi is a GM technique it is likely to meet significant opposition in various parts of the world, particularly in the EU which has possibly the strictest GM regulations (Davison, 2010). Alternatively, RNAi-mediated virus control could be delivered as a foliar spray (Wang *et al.*, 2011; Mulot *et al.*, 2018).

Other ways to prevent aphid colonisation are the use of physical barriers to prevent access to the crop e.g. horticultural fleeces, nets, or insect traps. However, these methods are unsuitable for large-scale crop production and do not provide further protection once a single aphid reaches the crop.

Control of TuYV in other countries is primarily by use of insecticides and there are no alternative control options other than those discussed for use in the UK

Breeding for aphid resistance and host resistance and/or tolerance

In view of the wide scale resistance of peach-potato aphid to insecticides, varieties of OSR that are either resistant or tolerant of TuYV are likely to provide the most successful means of combatting the virus. These have the advantage of reducing the chances of early TuYV infection which is likely to have the most detrimental effects on crops and will help varieties reach their true yield potential. A list of varieties currently available in the UK which show TuYV resistance are given in Table 16. These varieties possess the same source of resistance and hence are creating immense selection pressure for resistance breaking. There is a need to manage resistances by developing new resistant varieties where the resistance is based on other/new sources. As there is little or no resistance in OSR, these new sources of resistance will have to come from *Brassica oleracea* and *Brassica rapa*. The *Brassica oleracea* sources can also be exploited in UK vegetable brassicas (Walsh, J. Pers. Comm.).

Table 16. Varieties available in the UK showing TuYV resistance (as of summer 2019)

Variety	Company	Type	Status
Amalie	Limagrain	Conventional OP	National list
Ambassador	Limagrain	Restored hybrid	Candidate
Annalise	Limagrain	Conventional OP	National list
Architect	Limagrain	Restored hybrid	Recommended list
Artemis	Limagrain	Restored hybrid	Candidate

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Aspire	Limagrain	Conventional OP	Recommended list
Aurelia	Limagrain	Restored hybrid	Candidate
Temptation	DSV	Restored hybrid	Recommended list

An alternative approach to combat TuYV in OSR would be the use of R-genes that confer resistance to aphids. However, few of these have been reported and attempts at introducing aphid resistance into crops have had mixed success (Coleman, 2014). In general, aphid resistance appears to be polygenic although there are examples of single dominant R-genes (Dedryver *et al.*, 2010; Dogimont *et al*., 2010). Whilst effective, R-gene mediated resistance is often highly specific to a particular genotype/biotype and can be broken down in as little as two years after commercial release in the field (McDonald and Linde, 2002). However, these R genes can be stacked to make it harder for pests to evolve counter-resistance and to provide multiple resistances to different attackers.

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Virus management in carrots

Carrots and parsnips are grown mainly in East Anglia, the Midlands and the Lancashire mosses. A total of 11,933 hectares of carrots are grown in the UK, and 2,969 hectares of parsnips. This review follows on from, and supplements, the research conducted by Fox *et al*., 2011 and 2016 as part of the AHDB Horticulture funded projects FV382, '' Carrot: Symptomatic survey of virus complexes'' and FV382b ''The epidemiology of carrot yellow leaf virus-the development of a decision support system for the management of carrot viruses'', and Collier *et al*., 2016 as part of FV445 ''Carrots-optimising control of willow-carrot aphid and carrot whitefly''. The resulting factsheets from these projects are also available for further reading:

- AHDB Factsheet 07/16: Virus diseases of carrots
- AHDB Factsheet 01/16: Pest insects infesting carrot and other Apiaceous crops

Current viruses present in the UK

Plant Virus Online lists 83 viruses with the ability to infect members of the Apiaceae family, with 33 of these reported to infect carrot (Brunt et al., 1996; Adams *et al*., 2014). Around 12 of these are reported to occur in the UK. Table 17 lists eight of these viruses known to exist currently in the UK. This review will focus specifically on four key viruses which are the principle targets affecting carrot crops in the UK; namely *Parsnip Yellow Fleck Virus* (PYFV), the *Carrot Motley Dwarf Complex* (CMD) consisting of *Carrot red leaf virus* (CtRLV*), Carrot mottle virus* (CMoV) and *Carrot red leaf associated viral RNA* (CtRLVaRNA), and *Carrot Torrado Virus* (CaTV). A survey carried out by Fox *et al*., 2011 screened 35 carrot samples received at FERA, all showing foliar symptoms consistent with virus infection. 49% of the samples received contained at least one virus; CMoV was present in 65% of virus positive samples, with PYFV detected in only 3 of these samples. Of these samples where PYFV was detected, CtRLVaRNA was also present which was unexpected as this viral RNA is associated with transmission of the other viruses in the CMD complex. CMD was found to be associated with the development of necrotic root rot symptoms, however in some cases all three viruses in the complex were found and no visible symptoms observed; raising more questions than answers around the association of symptoms with CMD infection.

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Table 17. Summary of viruses reported on carrot in the United Kingdom, together with information on their mode of transmission and their vector. Viruses highlighted in bold italics are further discussed in the present Section.

Virus	Acronym	Genus	Mode of transmission	Vector
Parsnip Yellow Fleck virus	PYFV	Sequivirus	Aphid, semi-persistent, requires a helper virus (AYV)	*Cavariella aegopodii, C. pastinacae*
Carrot Motley Dwarf complex	CMD	CMD complex consists of three viral components: CMoV, CtRLV and CtRLVaRNA		
Carrot mottle virus	CMoV	Umbravirus	Aphid, persistent, requires a helper virus	*C. aegopodii*
Carrot Red Leaf virus	CtRLV	Luteovirus	Aphid, persistent	*C. aegopodii*
Carrot Red Leaf associated Viral RNA	CtRLVaRNA	Luteovirus	Aphid, requires a helper virus (CtRLV)	*C. aegopodii*
Carrot Torrado virus	CaTV	Torradovirus	Aphid	*C. aegopodii, M. persicae*
Carrot Yellow Leaf virus	CYLV	Closterovirus	Aphid	*C. aegopodii Myzus persicae*
Carrot closterovirus-1	CtCV-1	Closteovirus	Aphid	*C. aegopodii*
Heracleum latent trichovirus	HLV	Trichovirus	Aphid, semi-persistent, requires a helper virus (CYLV)	*C. aegopodii, C. pastinacae, C. theobaldi;*

Parsnip Yellow Fleck virus (PYFV)

PYFV was first reported in parsnip from the U.K by Murant and Goold (1968). Early infection can cause severe symptoms and death of individual plants. Infections occurring later in the season result in less severe symptoms such as mottling and discolouring of foliage with characteristic yellow flecks. Affected plants may develop secondary and/or misshapen roots while tops may die back (Fox *et al*., 2016). As discussed earlier, despite its relative importance as the main virus infecting carrots in the UK, of the 35 samples examined during FV382 (Fox *et al*., 2011), only three were found to contain PYFV; the exact reason for this finding remains unclear.

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Carrot Motley Dwarf complex (CMD complex)

CMD was first reported in carrot from the U.K. by Watson *et al*., 1964. CMD is caused by a complex of three viruses: *Carrot red leaf virus* (CtRLV), *Carrot mottle virus* (CMoV) and *Carrot red leaf virus associated RNA* (CtRLVaRNA) (King *et al*., 2012). Symptoms of CMD include reddening of leaves, mottling and is thought to cause a split in roots of carrot called 'kippering'. CMD is one of the most prevalent viruses in the UK; 35% of all samples and 65% of all virus positive samples tested during FV382 were found to contain CMoV. CtRLV is required for transmission of other viruses in the CMD complex; this will be discussed further later in the review.

Carrot Yellow Leaf virus (CYLV)

CYLV was first reported in carrot from Japan by Yamashita *et al*., 1976. The virus has been known to occur in the UK since the 1980s, though limited research has been conducted in the UK to date, due to its consideration as a minor issue to growers (Adams *et al*., 2014; Fox *et al*., 2016). Symptoms include an upright growth habit and yellowing of foliage in carrot with no visible symptoms in wild Apiaceae species. Yellowing of foliage is a common feature of other viruses which infect carrot, so in some cases where multiple infections have occurred, it is difficult to pin point the exact cause of the symptoms. Research conducted during FV382a found that CYLV infection was strongly associated with carrot internal necrosis.

Carrot torrado virus (CaTV)

CaTV1 was first detected from carrots in the United Kingdom (Rozado-Aguirre *et al*., 2016) following a NGS study to investigate internal vascular necrosis in carrot. CaTV belongs to a recently discovered group of viruses, the torradoviruses (van der Vlugt *et al*., 2015) and is the first of this group to be found in the UK. It is also the first member of this group to be found affecting root crops. The virus was subsequently detected in France by (Rozado-Aguirre *et al*., 2017). CaTV is not thought to cause any observable symptoms in carrots, but may contribute to yield reduction (Fox *et al*., 2016). Because it is such a recently discovered virus in the UK, its relative incidence and impact on the UK carrot industry remains to be, and is currently being fully determined.

New emerging viruses

Table 18 lists the current viruses known to infect carrot according to Plant Virus Online (Brunt et al., 1996), but have not yet been detected from carrot crops in the United Kingdom. In the last 2 years, there have been a number of new disease reports concerning viruses infecting both carrot and wild Apiaceae species, including identification of *Arctopus echinatus associated virus* (AeaV), isolated from *Arctopus echinatus,* a perennial weed of the Apiacae

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family in South Africa (Richet *et al*., 2018), the first report *of Apium Virus Y* and *Carrot Thin Leaf Virus* in Parsley in Slovenia (Mehle *et al*., 2019) and the first report of *Carrot torrado virus* 1 and *Carrot thin leaf virus* from the wild Apiacae species *Torilis arvensis* ssp. *arvensis* in Greece (Lotos *et al*., 2018).

Table 18. Viruses which are known to infect carrots, but have not yet been identified in carrot crops in the United Kingdom.

Virus	**Acronym**	**Genus**
Alfalfa mosaic virus	AMV	Alfamovirus
Arabis mosaic virus	ArMV	Nepovirus
Beet pseudo-yellows virus	BPYV	Closterovirus
Carrot latent virus	CtLtV	Nucleorhabdovirus
Carrot mosaic virus	CtMV	Potyvirus
Carrot mottle mimic virus	Unknown	Umbravirus
Carrot temperate 1-4 virus	CTeV-1 to 4	Cryptovirus
Carrot thin leaf virus	CTLV	Potyvirus
Cassava green mottle virus	CGMV	Nepovirus
Celery mosaic virus	CeMV	Potyvirus
Celery yellow net virus	Unknown	Unknown
Clover yellow vein virus	ClYVV	Potyvirus
Coriander feathery red vein virus	CFRVV	Nucleorhabdovirus
Cucumber mosaic virus	CMV	Cucumovirus
Galinsoga mosaic virus	GaMV	Carmovirus
Lettuce infectious yellows virus	LIYV	Closterovirus
Oat blue dwarf virus	OBDV	Marafivirus
Okra mosaic virus	OkMV	Tymovirus
Parsnip mosaic virus	ParMV	Potyvirus
Poplar mosaic virus	PopMV	Carlavirus
Potato black ringspot virus	PBRsV	Nepovirus
Tobacco ringspot virus	TRSV	Nepovirus
Tomato black ring virus	TBRV	Nepovirus
Tulip X virus	SqVYV	Potexvirus
Strawberry latent ringspot virus	SLRSV	Nepovirus

Vectors and alternate hosts of key viruses

Vectors of key viruses

Parsnip Yellow Fleck virus (PYFV)

PYFV is transmitted by *C. aegopodii* and *C. pastinacae* in a semi-persistent manner. It is not transmitted by *C. theobaldii*. The virus is lost by the vector when it moults and it is not transmitted congenitally to the progeny of the vector. PYFV is transmitted via the aphids

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mouthparts and is stored in the foregut. Transmission is relatively quick, with the vector needing only 10–15 minutes to take up the virus and only two minutes feeding to infect a healthy plant. PYFV requires a helper virus, *Anthriscus yellows virus* (AYV) for vector transmission. If a plant is infected by both viruses, feeding aphids can pick up one or both viruses and transmit them. However, if AYV is not present onward transmission will not occur. PYFV can infect carrot, however, carrot is not susceptible to AYV. Thus PYFV is not serially transmitted in carrot crops and PYFV infections have come from another plant host susceptible to both viruses; likely another wild Apiaceae species. PYFV can be transmitted by mechanical inoculation but is not transmitted by seed or pollen.

Carrot Motley Dwarf complex (CMD complex)

Viruses triggering CMD are transmitted by *C. aegopodii* in a persistent, non-propagative manner (Elnagar and Murant, 1978; Watson *et al*., 1964; Watson *et al*., 1998). Both CMoV and CtRLVaRNA cannot produce the coat protein necessary for transmission and so they need another virus present to be passed on by aphids. In coinfections with CtRLV, some of the CtRLV virus particles carry the CMoV and CtRLVaRNA genetic material allowing all three viruses to be transmitted simultaneously, or occasionally by chance just one or two viruses may be passed on. *M. persicae*, *C. pastinacae*, *C. theobaldi* and several other aphid species have been tested for their ability to transmit CtRLV and CMoV, both components of the CMD complex but no transmission has been documented (Murant, 1974; Stubbs, 1952; Waterhouse, 1985; Watson *et al*., 1964). Naseem *et al*., 2016 examined the involvement of *M. persicae* in transmission of the three components of the CMD virus complex and causation of CMD in plants of the Apiaceae family. *M. persicae* was found to have similar capability to transmit viruses associated with CMD as that of *C. aegopodii*. This enhanced virus transmissibility may be explained by the use of purified virus preparation in the study by Naseem *et al*., (2016) compared to plant sap extracts used in previous studies (Weber and Hampton, 1980). A study by Gungoosingh-Bunwaree *et al*., 2009 confirmed the presence of CMoV by sap transmission to *N. benthamiana* and *N. occidentalis* 'P1'. CMoV is transmissible via mechanical inoculation (Elnagar and Murant, 1978; Watson *et al*., 1964) but is not transmitted by seed or pollen. Though carrot viruses are not thought to be seed transmitted, CtRLV has been found previously during export certification testing. Mechanical transmission of CtRLVaRNA is not known (Falk *et al*., 1999).

Carrot Yellow Leaf virus (CYLV)

CYLV is transmitted by *C. aegopodii*, *C. pastinacae* and *C. theobaldi* in a semi-persistent manner. Transmission work carried out in FV382 also demonstrated the ability of *M. persicae* to transmit the virus. CYLV is transmitted in a similar mechanism as PYFV, where the virus

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is sucked into the foregut of the aphid vector and can be rapidly transmitted to a new host. CYLV does not require a helper virus for transmission, however Bem and Murant (2008) and Adams *et al*., 2014 report that it can help the transmission of Heracleum latent closterovirus. In FV382, CYLV was observed to transmit at a very low (0.5% or less) efficiency, with the rate of transmission greater from weed to carrot than between carrots and present in a wide range of apiaceous weed hosts such as hogweed (Bem and Murant, 1979). CYLV can be transmitted by mechanical inoculation.

Carrot torrado virus (CaTV)

CaTV is transmitted by both *C. aegopodii* and *M. persicae*, with the latter proving to be a more efficient vector across the three tested plant species (carrot, *N. benthamiana* and chervil). CaTV does not require a helper virus for transmission (Rozado-Aguirre *et al*., 2016). This study by the former authors was the first report of aphid transmission of a member of the Torradovirus genus. In recent testing of carrot seed lots at FERA, two out of 10 seed lots tested contained CaTV. Although the infection rate was at a very low level, and seed transmission has not yet been demonstrated, there is clearly the potential for seed to be a source of virus infections.

Table 19. Summary of known vectors of the key viruses infecting carrot crops detailed in this review-PYFV, CYLV, CaTV and the CMD complex.

Virus	**Vector**
PYFV	*C. aegopodii, C. pastinacae,*
CYLV	*C. aegopodii, M. persicae*
CaTV	*C. aegopodii, M. persicae*
CMoV	*C. aegopodii*
CtRLV	*C. aegopodii*

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Alternate host species of key viruses

Parsnip Yellow Fleck virus (PYFV)

In survey work carried out during Defra IF0188 and HortLINK HL0149, PYFV was mostly found in cow parsley and occasionally in hogweed. AYV was also found most commonly in cow parsley. The results of genetic analyses conducted during both HL0149 and IF088 strongly suggest that most PYFV infections found in carrot crops originate from cow parsley. Plant Virus Online also lists chervil, spinach, globe amaranth, *Chenopodium quinoa, Montia perfoliata, C. amaranticolor and Nicotiana clevelandii* as alternate hosts for PYFV (Brunt et al., 1996) for PYFV.

Carrot Motley Dwarf complex (CMD complex)

The 2012 survey conducted during FV382b found that the CMD complex of viruses were present in hogweed, cow parsley and rough chervil. Plant Virus Online also lists common bean, coriander, *C. quinoa and N. clevelandii* as alternate hosts for CMoV Chervil, *Apium leptophyllum* and coriander for CtRLV (Brunt et al., 1996).

Carrot Yellow Leaf virus (CYLV)

To date, there has been no large scale surveillance studies carried out to look at the importance of different sources for CYLV. In a small scale survey carried out in 2016 during FV382b, CYLV was only present in samples of cow parsley and not present in hogweed or rough chervil. Plant Virus Online lists chervil, coriander, *Pimpinella anisum, N. benthamiana and N. clevelandii* as alternate hosts for PYFV (Brunt et al., 1996).

Carrot torrado virus (CaTV)

CaTV was not identified in any of the weed samples tested by FERA in 2016 as part of FV382b, including wild apiaceous hosts such as hogweed, cow parsley, hemlock and rough chervil suggesting that infection could come from another source, likely either from previous carrot crops overwintered in the field or from seed sources. A recent paper from Lotus *et al*., 2018 reports the weed species *Torilis arvensis* subsp. *arvensis* (family Apiaceae), from Greece as a natural host of the isolate CaTV1.

Table 20. Summary of known alternate host species of the key viruses infecting carrot crops detailed in this review-PYFV, CYLV, CaTV and the CMD complex.

Virus	Alternate host species
PYFV	Cow parsley, hogweed, chervil, spinach, globe amaranth, *Chenopodium quinoa, Montia perfoliata, C. amaranticolor, Nicotiana clevelandii*
CYLV	Chervil, coriander, *Pimpinella anisum, N. benthamiana, N. clevelandii*
CaTV	Chervil, *N. benthamiana*, *Torilis arvensis* subsp. *arvensis*
CMoV	Common bean, coriander, *C. quinoa, N. clevelandii, N. benthamiana and N. occidentalis*
CtRLV	Chervil, *Apium leptophyllum*, coriander

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Detection and Identification Methods Based on Biological Properties

Parsnip Yellow Fleck virus (PYFV)

PYFV can be detected by its density in different solvent solutions; in caesium chloride (CsCl) it has a density of 1.297-1.49 g cm^{-3}. When measuring its optical density, PYFV has an A260/A280 ratio of 1.46-1.59. Virions of PYFV are found in the mesophyll; they are isometric and ca. 31 nm in diameter. ELISA for PYFV are commercially available, with a DAS-ELISA test using antisera raised against PYFV from spinach first developed in 2001 by Morgan *et al*., 2004.

Carrot Motley Dwarf complex (CMD complex)

Both CtRLV and CMoV, components of the CMD complex can be characterised by their density in a solvent solution. The density of CtRLV and CMoV is 1.403 g cm^{-3} and 1.15 g cm^{-3} in CsCl respectively. No density could be found for CtRLVaRNA. Leaf sap of CtRLV contains few virions; virions present are isometric and non-enveloped, ca. 25 nm in diameter and rounded or angular in profile. Virions of CtRLV can be found in the phloem and companion cells; in the cytoplasm and in cell vacuoles. Virions of CMoV are unusually shaped and not fully identified. No information could be found on characterisation of virions of CtRLVaRNA using physical methods or microscopy. ELISA is available for detection of CtRLV (Watson and Falk, 1994; Gungoosingh-Bunwaree *et al*., 2009) and CMoV (Watson and Falk, 1994).

Carrot Yellow Leaf virus (CYLV)

Leaf sap of CYLV contains few virions; the virions present are filamentous, non-enveloped; and usually flexuous with a clear modal length of 1650 nm and 12 nm wide. Virions can be found in the leaves and phloem of infected plants, both in the cytoplasm and the nuclei (Yamashita *et al*., 1976). Koch's Postulates could not be demonstrated for CYLV in FV382 due to failure to transmit CYLV to a secondary host.

Carrot torrado virus (CaTV)

CaTV is a relatively new discovery; no information on this virus is available through Plant Virus Online and with the exception of the studies conducted by Rozado-Aguirre *et al*., 2017 and Tokuda *et al*., 2019, no public information could be found on the biological properties of CaTV. To the authors' knowledge no ELISA or other diagnostic assays are available for CaTV.

Detection and Identification Methods Based on Viral Nucleic Acid

Detection of carrot viruses is currently carried out using conventional PCR methods, coupled with the use of degenerate primer sets which allows for detection a number of pathogens of

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the same genus (Adams *et al*., 2009). However, such targeted testing or even multi-target approaches such as micro-array based methods are unlikely to reveal the presence of unknown or new viruses, unless cross-hybridisation to known close relatives occurs. Next generation sequencing (NGS) is now successfully in use for the detection of novel viruses (Barba *et al*., 2014; Barzon *et al*., 2011) or for the diagnosis of unusual strains of plant viruses (Roossinck *et al*., 2015,) Going forward, this approach is fast becoming more cost effective as high throughput platforms for both sequencing and downstream data processing and bioinformatics develop.

Parsnip Yellow Fleck virus (PYFV)

Virions of PYFV contain 40% nucleic acid. Its genome is unipartite and consists of ssRNA with a total genome size of 9.871 kb (Turnbull-Ross *et al*., 1993). The first complete nucleotide sequence of PYFV was determined for isolate P-121 (a parsnip serotype) by Turnbull-Ross *et al*., 1992. RT-PCR methods for PYFV detection are well established, with TaqMan probes first developed in the U.K. by North *et al*., 2004.

Carrot Motley Dwarf complex (CMD complex)

As stated previously, both CMoV and CtRLVaRNA, are capable of replicating independently but are dependent on CtRLV for their transmission to new plant hosts. This is because CMoV and CtRLVaRNA do not form a coat protein and are encapsidated by the CtRLV coat protein (Falk *et al*., 1999; Murant, 1974; Murant *et al*., 1985; Waterhouse and Murant, 1983). Genomic nucleic acid of CtRLV was originally isolated by Murant *et al*., 1985. Virions of CtRLV have been found to contain 28% nucleic acid, 72% protein and 0 % lipid. The genome is unipartite and consists of linear, ssRNA with a total genome size of 5.75 kb. The genome of CMoV is unipartite and consists of linear, ssRNA with a total genome size of 4.75 kb. Replication of either CtRLV or CMoV does not depend on a helper virus. No information could be found on CtRLVaRNA.

RT-PCR methods are available for distinguishing the viruses associated with CMD (see Morton *et al*., 2003; Gungoosingh-Bunwaree *et al*., 2009 and Tang *et al*., 2009); the first study was presented by Vercruysse *et al*., 2000 where RT-PCR assays were optimised for the simultaneous detection of the three viruses in infected parsley and chervil plants and in individual viruliferous aphids. Recently, Naseem *et al*., 2016 showed by RT-PCR that *M. persicae* exposed to CMD-infected chervil plants transmitted CtRLV, CMoV and CtRLVaRNA to disease free chervil, fennel, celery, carrot, coriander and parsley. Sequence analysis of the amplified virus genes showed high sequence diversity with corresponding sequences available in GenBank.

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Carrot Yellow Leaf virus (CYLV)

CYLV is a ssRNA virus with a genome size of 16.3 kb (Menzel *et al*., 2009). Adams *et al*., 2014 and Fox *et al*., 2016 describe the use of RT-PCR using gene specific primers for diagnostic testing of CYLV, as well as CtCV-1. Adams *et al*., 2014 used Illumina sequencing to characterise CYLV as the potential cause of carrot root necrosis. Of the 102 carrots with necrotic symptoms tested for the presence of CYLV by RT-PCR, 99 samples (98%) tested positive for CYLV. During FV382 (Fox *et al*., 2016), all biological material tested for virus presence including plants used during transmission tests, weed samples collected from around the UK, as well as samples of carrot seed were subjected to this diagnostic testing procedure.

Carrot torrado virus (CaTV)

CaTV1 possesses icosahedral particles and single-strand positive-sense bipartite RNA genomes designated RNA1 and RNA2 (Tokuda *et al*., 2019). The complete nucleotide sequence of one CaTV1 isolate from the United Kingdom has been determined by (Rozado-Aguirre *et al*., 2017). CaTV1 genome sequences were obtained from a previous NGS study conducted by (Adams *et al*., 2014), then compared to other members and tentative new members of the genus. Rapid amplification of cDNA ends (RACE) was used to amplify and sequence each end of RNA1 and RNA2. RNA1 was found to encode the proteins involved in virus replication, and RNA2 encodes the encapsidation and movement proteins. Analysis of nucleotide sequence and coat protein regions confirmed that CaTV1 should be classified as a member of a new species within the genus Torradovirus.

Rozado Aguirre *et al*., 2016 demonstrated that both RT-PCR and RT-qPCR successfully amplified and detected CaTVRNA1 and RNA2 in infected samples, using previously published Torradovirus genus specific RT-PCR primers (Verbeek *et al*., 2012). The strength of the assays was confirmed by different people performing them on different days in different PCR machines. The presence of CaTV RNA1 and RNA2 was later confirmed in France using an RT33 qPCR and two pairs of specific primers (Rozado-Aguirre *et al*., 2016). To confirm the results, four of the CaTV1 positive samples were then further tested by two-step RT-PCR assays using Torradovirus generic primers specific for each of the genomic RNAs (Verbeek *et al*., 2012). The product of the RT-PCR was directly sequenced to provide further confirmation of its identity; the nucleotide and amino acid identity of this French isolate was found to be significantly different from the UK isolate of CaTV.

RT-PCR methods can also be used on seed batches, with Fox *et al*., 2016 successfully identifying CaTV from 2 seed lots out of 10 tested. Interpretation of the virus content in the batches was carried out using the ISTA programme SeedCalc8

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(https://www.seedtest.org/en/links-statistical-links-for-seed-analysts-_content---1--3425--271.html). The most recent study of CaTV genomics comes from Tokuda *et al*., (2019) who detected a CaTV1 isolate, designated CaTV1-J from *Angelica keiskei* (family Apiaceae), a perennial herb used for food and medicine in Japan, and determined its complete genome using Illumina sequencing.

Novel technologies

No specific information was discovered on the use of robotics or aerial imagery for the detection of viruses in carrot crops.

Modelling

Decision support systems for carrot growers in the UK are centred around aphid monitoring networks provided by AHDB, Warwick University and Rothamsted Research and aphid predictions based on average winter temperatures. Winter temperature is the dominant factor affecting aphid migration phenologies for *M. persicae* with a 1°C increase in average winter temperature advancing aphid migrations by 4-19 days depending on the species (Zhou *et al*., 1995). This research became central for aphid migration predictions in the UK via Warwick University, AHDB and Rothamsted Research and plays a major part in informing UK carrot growers of the aphid and aphid-borne virus risk.

Willow-carrot aphids (*C. aegopodii*) are captured in the network of suction traps operated by the Rothamsted Insect Survey. They can also be captured in yellow water traps and commercial monitoring services using water traps are available. A forecast system developed at the University of Warwick is based on accumulated day-degrees (D°) from 1st February (base 4.4°C). Information from the Rothamsted Suction trap captures at Wellesbourne and Kirton is used to estimate the mean number of D° until the first aphid of the year is caught in a suction trap (the start of the migration to carrot). This is after approximately 360D°. The output from this program is currently available as part of the AHDB Pest Bulletin which is hosted on the Syngenta UK website (https://www.syngenta.co.uk/ahdb-pest-bulletin). For peach-potato aphid (*M. persicae*) monitoring in early March, the Rothamsted Insect Survey produces a forecast of the timing of the migration and the likely relative abundance of peach-potato aphids which can be expected in the early summer. This is based on winter temperatures.

Chemical and cultural management options

The principles of virus management as detailed by Fox *et al*., 2017 in AHDB Horticulture FV 453 are applicable across multiple crops and cropping scenarios. In this review, the section on potato virus management details the use of straw mulching, crop borders and mineral oil

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spraying for effective management of viruses and their associated vectors on seed and ware potatoes and the section on OSR discusses the suitability of biopesticides and physical barriers such as fleeces, nets, or insect traps for vector management. However, in the case of carrots, often the management options are not agronomically feasible. In-field storage of carrots in the UK could be creating a bridge for carrot-to-carrot transmission between seasons. The distribution of carrot growing areas in the UK means that isolation of crops, or breaks in growing, are not an option to reduce exposure to virus infection. Removal of weed hosts is often not possible as they are so widespread. Seed may also be an important virus source.

Thus the primary method of virus management in carrots is mainly through insecticide use and effective aphid management strategies. Current aphid control measures are based around spirotetramat and flonicamid, aimed primarily against PYFV with intensive spray programmes through May and June. Bayer have recently registered Sivanto (flupyradifurone) in the Netherlands. This is a butenolide – a new class of insecticides, which acts on the central nervous system of insects. Although not a neonicotinoid, it could be positioned similarly in terms of rapid efficacy, quick knock-down and positive effects on virus transfer. This active has possibilities to be available in carrots. It is likely to be limited to only 1 x application per year per crop. Requiem a biological-type insecticide based on the terpenoid blend QRD460 is also in the registration process in the Netherlands, initially for protected crop uses, but could be looked at on outdoor crops too. With a zero residue profile, it is mainly targeting small sucking pests, so includes some aphid species, some mites and some thrips. It has a relatively rapid knock-down effect, but does not persist on the leaf surface (Lacey, T., Pers. Comm.). Syngenta acquired DevGen in 2013 to develop RNAi as a sprayable crop protection product to control insects; this could be a future tool for virus management, the company are also developing a biostimulant that helps elevate the effect of viruses if infection occurs. Both products are currently confidential (Newbert, M. Pers. Comm.).

Timing of sprays is generally linked to regional and local aphid trapping. Trials in 2015 during FV 445 (Collier *et al*., 2016) showed that thiamethoxam seed treatment was the most effective product at reducing aphid infestations for early season control; there are also indications that this treatment may reduce virus transmission compared with foliar spray treatments. Thereafter there is a shortage of foliar aphicide sprays to cover the mid to late season period. Spirotetramat or thiacloprid can be used; pyrethroids are less effective against willow-carrot aphid than some other treatments while some peach-potato aphids are resistant to pyrethroid insecticides. These aphicide resistance issues coupled with the withdrawal of key active ingredients such as thiamethoxam and pirimicarb complicates possible chemical control strategies, and resistance management should also be taken into account when developing

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spray programmes. Several insecticides used on carrots are effective against more than one pest species and so careful selection of insecticide treatments based on the life cycles of all potential pests is essential.

For non-chemical control of aphid vectors, crop rotation and particularly spatial separation of new crops from previous sources of infestation will reduce pest and virus damage. The use of net covers may be beneficial for the control of all pest species, if deployed at the appropriate time and with a suitable mesh size. Sticky traps placed in suitable areas to assess field populations of different aphids accurately should also be used. Natural predators such as ladybirds and parasitoid wasps can be very effective in reducing aphid infestations on carrot in some years though it is not known what impact this may have on transmission of virus. Where feasible pesticides and/or methods of application should be chosen carefully to protect beneficial species.

Elsewhere in the world; in Australia, severe infections of CMD complex first appeared in carrots in the 1940s and 50s, however by the mid -1960s the virus was well controlled through the selection of virus tolerant or aphid repellent varieties, a change in sowing dates to avoid seasonal flushes of the carrot aphid, widespread use of insecticides on carrots and the effectiveness of an introduced parasite of *C. aegopodii* (Buchen-Osmond *et al*., 1988). Reflective mulches such as aluminium foil and coloured plastic mulches have been shown to be effective in controlling virus diseases in various crops (Jones and Chapman 1968, Lobenstein *et al*., 1975; Daiber and Donaldson 1976) by reducing the number of insect vectors or by making the habitat less suitable for the insect (Cohen, 1984). Cardona *et al*., 1981 demonstrated that rice-straw mulch gave effective control of the leafhopper *Empoasca kraemeri* Ross in dry beans. Zalom (1981) demonstrated that aluminium-foil mulch gave effective control of the aster leafhopper and aster yellows in head lettuce. Results from Setiawan and Ragsdale, (1987) demonstrated that aluminum-foil and straw mulches gave control of aster leafhoppers and aster yellows in carrots equal to that of a conventional insecticide spray program.

Breeding for aphid resistance and host resistance and/or tolerance

Carrot is an allogamous crop with strong inbreeding effect (Pitrat, 2012). Modern varieties such as Nairobi are more homogeneous F1 or three-way cross hybrids produced with a cytoplasmic male sterility. Data on the susceptibility of a range of varieties to different viruses is very limited and currently based upon casual observations rather than quantitative data. To date, the majority of work carried out on carrot viruses has been carried out on cv. Nairobi as this is the variety most commonly grown in the UK and the variety in which root necrosis was first identified. Partial resistance to *Carrot virus Y* (Jones *et al*., 2005) and to CMD complex

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(Watson and Falk, 1994) has been described. Jones *et al*., 2005 screened 34 accessions of wild carrot germplasm and 16 other *Daucus* spp. for resistance to CarVY by inoculating with infective aphids. Symptom severity varied widely among accessions but no source of extreme resistance to CarVY was found.

Genetic modification and CRISPR technology

Virus resistance can potentially be introduced into plants through post transcriptional gene silencing (PTGS), through the introduction of partial RNA viral sequences into the plant genome in a double stranded form (Waterhouse *et al*, 1998; Wang *et al*., 2000; Zraycha *et al*., 2007; Frizzi *et al*., 2010). Maheswaran, 2006 describes the transformation of carrot cells with the *Carrot virus Y* (CarVY) pPOPOV-CarVY resistance construct via A*grobacterium tumefaciens* mediated transformation, regeneration of selected carrot lines containing the resistance construct and the assessment of these transgenic lines for virus resistance by inoculation with CarVY under glasshouse conditions. A transformation efficiency rate of 0.85% was achieved for cv. Crusader using the commonly used AGL-1 strain of *A. tumefaciens*; six transgenic carrot plants of this cultivar containing the transgene encoding the CarVY resistance sequence were produced. All of these plants showed a degree of resistance to CarVY. Importantly, the authors showed that only a small region of ca. 300 nucleotides in length of the viral genome was required to confer resistance to the virus. From an environmental perspective, this is highly desirable as it minimises the risk of viral recombination between transgenic and non-transgenic plants in the field, should genetically modified crops ever be commercialised in the UK and beyond.

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Virus management in peas

This review follows on from, and supplements the thoroughly comprehensive review conducted by Fox *et al*., 2017 as part of AHDB Project FV 453. The findings, conclusions and recommendations from that study acted as a catalyst for the AHDB research project FV 459 on improving our understanding of pea viruses in the UK; a collaborative project involving PGRO and FERA.

Current viruses present in the UK

Fox *et al*., 2017 states that 124 viruses are reported to have the ability to infect peas on Plant Virus Online (Brunt *et al*., 1996), however only 43 of these occur from natural infections with the remainder listed as experimental hosts. Table 21 lists the seven pea viruses known to exist currently in the UK. This review will focus specifically on three key viruses; namely *Pea seed-borne mosaic virus* (PSbMV), *Bean leaf roll virus* (BLRV) and *Bean yellow mosaic virus* (BYMV).

Table 21. Summary of viruses reported on peas in the United Kingdom, together with, where available, information on their mode of transmission and their vector. Viruses highlighted in bold are further discussed in the present Section.

Virus	Acronym	Genus	Mode of transmission	Vector
Pea early-browning virus	PEBV	Tobravirus	Nematode	*Paratrichodorous anemones, P. pachydermus, Trichodorous primitivius, T. viruliferous*
Pea seed-borne mosaic virus	PSbMV	Potyvirus	Aphid; non-persistent Wind mediated	*Macrosiphum euphorbiae, Myzus persicae, Acyrthosiphon pisum, A. craccivora, Aphis fabae, Dactynotis escalanti, Rhopalosiphum padi*
Bean leaf roll virus	BLRV	Luteovirus	Aphid; persistent	*A. pisum, A. craccivora, M. persicae*
Bean yellow mosaic virus	BYMV	Potyvirus	Aphid; non-persistent, seed transmission reported but uncommon	*A. pisum, M. euphorbiae, M. persicae, A. fabae*
Pea enation mosaic virus	PEMV	Nepovirus	Aphid; persistent	*A. pisum, M. euphorbiae, M. persicae*
Pea Streak Virus	PeSV	Carlavirus	Aphid; non-persistent	*A. pisum*
Broad bean true mosaic virus	BBTMV	Cornovirus	Weevils	*Sitona lineatus, Apion vorax*

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Pea seed-borne mosaic virus (PSbMV)

PSbMV was first reported in pea by Musil, 1966 and is a member of the Potyvirus family. The severity and type of PSbMV expression in pulse crops is influenced by cultivar, environment, and virus pathotype (Rashedetal *et al*., 2018). Infected pea plants experience symptoms such as mosaic leaves, downward or upward leaf curling, thickened and tightly curled tendrils, shortened internodes, stunting of plant canopy, chlorosis, terminal rosetting of flower structures and vein clearing (Mink *et al*., 1969; Larsen 2001). Pea plants infected with PSbMV produce seed which has split seed coats, discolourations and reductions in seed weight (Coutts *et al*., 2009). These symptoms can cause downgrading and/or rejection of seed (Coutts *et al*., 2008).

Bean leaf roll virus (BLRV)

BLRV was first reported in pea and bean from Germany by Quantz and Volk, 1954. BLRV is a member of the Luteoviridae and is distributed worldwide. Following a two to four week incubation period, initial BLRV symptoms are expressed as interveinal chlorosis, upward rolling of the fully expanded leaves and reduced pod numbers, resulting in yield losses of up to 80% (Heathcote and Gibbs 1962; Rashedetal *et al*., 2018). Generally, BLRV infections that occur at the later stages of plant development are less damaging than early occurring infections (Bos *et al*., 1988). BLRV yellowing and mosaic symptoms are similar to those caused by water stress, nutrient deficiency, root rot diseases, and other viruses.

Bean yellow mosaic virus (BYMV)

BYMV was first reported in pea by Doolittle and Jones, 1925. Symptoms in pea appear as vein clearing, random dark green patches on leaves and more leaf mottling than a mosaic (Larsen 2001). Though not considered a major pathogen of peas, its importance lies in its broad host range, with 35 plant genera, from 11 families reported as BYMV hosts, including Fabaceae (Rashed *et al*., 2018).

New emerging viruses

Fox *et al*., 2017 discuss the newly emerging group of Nanoviruses as potentially damaging to UK field pea crops: in particular the new virus *Pea necrotic yellow dwarf virus* (PNYDV) which has spread through Germany, the Netherlands and Austria, with high yield losses reported in pea and faba bean in Austria (Gaafar *et al*., 2016, 2017). The most recent reports come from Ahsan and Ashfaq, 2018, who demonstrated the first *Cucumber Mosaic Virus* (CMV) subgroup II infecting pea in Pakistan, and Gaafar *et al*., 2017 described the first report of *Pea necrotic yellow dwarf virus* (PNYDV) in The Netherlands.

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Vectors of key viruses

Pea seed-borne mosaic virus (PSbMV)

PSbMV is transmitted in a non-persistent manner (Pirone and Harris, 1977).The main transmission route is seed, with up to 100% transmission rate in pea, especially when testa are split. Although mainly spreading via infected seed, aphids are also a vector for PSBMV, with *A. pisum*, *A. craccivora*, *A. fabae*, *D. escalanti*, *M. crataegarius*, *R. padi* all reported as potential transmitters. It can also be transmitted by mechanical inoculation (Congdon, 2017) investigated the PSbMV transmission efficiencies of five aphid species previously found landing in south-west Australian pea crops in which PSbMV was spreading. Differing aphid transmission efficiencies were observed between cultivars that were designated as PSbMV susceptible; the transmission efficiencies of *A. craccivora*, *M. persicae*, *A. kondoi* and *R. padi* were found to be 27%, 26%, 6% and 3%, respectively. With plants of a partially PSbMV resistant pea cv. PBA Twilight, transmission efficiencies of *M. persicae, A. craccivora and R. padi* were 16%, 12% and 1%, respectively, reflecting putative partial resistance to aphid inoculation. To examine aphid alighting preferences over time, free-choice assays were conducted with *M. persicae* and *R. padi*; efficient and inefficient vector species, respectively. *R. padi* alatae exhibited a general preference for PSbMV-infected pea and faba bean plants after 30 min–4 h, but preferred mock-inoculated plants after 24h. In contrast, *M. persicae* alatae alighted on mock-inoculated pea plants preferentially for up to 48h following their release. Higher numbers of volatiles representing a range of compound groups such as aldehydes, ketones and esters were found in the headspaces of PSbMV-infected than of mock-inoculated pea or faba bean plants, indicating that PSbMV induces physiological changes in these hosts which manifest as altered volatile emissions. These alterations could be responsible for the differences in alighting preferences.

Bean leaf roll virus (BLRV)

BLRV shows high levels of vector specificity and is transmitted by *Acyrthosiphon pisum, M. persicae, M. euphorbiae, Megoura viciae* and *A. craccivora* in a persistent manner (Ashby, 1984). However, in a study by Ortiz and colleagues (2005), *A. fabae, A. craccivora*, and *M. persicae* failed to transmit the virus successfully to uninfected broad bean, despite the aphids testing positive for the pathogen, which suggests that these species may not be as efficient vectors as *A. pisum*. BLRV infection can help the vector transmission of another virus called bean yellow vein banding umbravirus. BLRV can be transmitted by grafting but is not transmitted by mechanical inoculation and is not sap, pollen or seed transmissible.

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Bean yellow mosaic virus (BYMV)

BYMV is an aphid-transmitted non-persistent virus with a wide vector and host range (Parrella and Lanave 2009). Vectors of BYMV include *A. pisum, M. euphorbiae, M. persicae* and *A. fabae*. BYMV can also be transmitted by mechanical inoculation and is transmissible by seed albeit at a low rate, estimated at around 3% (Bos *et al*., 1988).

Future potential for resistance developments in Myzus persicae

Due to stringent management techniques, R3 resistance, or extreme/high levels of resistance has not existed in UK populations of *M. persicae* for the last 5 years or so, However glasshouse imports of overseas *M. persicae* could result in it arriving back to the UK. This is relevant as organophosphates are no longer in use anymore in the UK, except in select cases, and the range of available actives and modes of action are in steady decline. Modelling resistance loss when modes of action are removed could be a potentially useful tool for resistance management strategies in the future.

Table 22. Vectors of key viruses in detailed in this review

Virus	Vector
PSbMV	*M. euphorbiae, M. persicae, A. pisum, A. craccivora, A. fabae, D. escalanti, R. padi*
BLRV	*A. pisum, A. craccivora, M. persicae*
BYMV	*A. pisum, M. euphorbiae, M. persicae, A. fabae*

Alternative hosts of key viruses

Pea seed-borne mosaic virus (PSbMV)

PSbMV infects a number of hosts including lentil (*Lens culinaris* M.), chickpea (*Cicer arietinum L*.), broad bean (*Vicia faba* L.), shepherd's purse (*Capsella bursa-pastoris* L.), black medic (*Medicago lupulina* L.), and alfalfa (*Medicago sativa* L.) (Fox *et al*., 2017; Beck *et al*., 2018). Aftab *et al*., 2018 report the detection of PSbMV from naturally infected fenugreek in Australia, thus identifying a new host for PSbMV. Fortunately, the virus was not found to be seed borne and was not detected in symptomless plants. Though fenugreek is not yet grown in the UK, it provides scope for other annual, herbaceous legume crops to become infected with this virus, thus acting as a potential reservoir and alternate hosts for PSbMV. Plant Virus Online also lists *Chenopodium quinoa* and *C. amaranticolor* as alternate hosts for PSbMV (Brunt et al., 1996).

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Bean leaf roll virus (BLRV)

The host range of BLRV is limited to the Fabaceae family; Plant Virus Online lists Alfalfa (*Medicago sativa*), White clover (*Trifolium repens*), broad bean (*Vicia faba*), common bean (Phaseolus vulgaris), chickpea (*Cicer arietinum*), Cowpea (Vigna unguiculata) and lentil (*Lens culinaris*) as alternate hosts for BLRV (Brunt et al., 1996).

Bean yellow mosaic virus (BYMV)

BYMV has a wide alternate host range of both wild and cultivated species; Plant Virus Online lists Common bean (*Phaseolus vulgaris*), *Gladiolus sp., Freesia sp., Lupinus luteus, Robinia pseudoacacia, Trigonella foenum-graecum, Vicia sativa, Papaver somniferum , Arachis hypogaea, Crotalaria spectabilis,* Soybean (*Glycine max*), *Canna spp., Trifolium subterraneum, Eustoma russellianum*, broad bean (*Vicia faba*), alfalfa (*Medicago sativa*), *Trifolium hybridum, T. vesiculosum, T. incarnatum, T. pratense and T. repens* as alternate hosts for BYMV (Brunt et al., 1996). Wang *et al*., (2019) provide the most recent report of the first case of BYMV infection in nasturtium in Hawaii. Nasturtium is a widely grown herbaceous ornamental in Hawaiil the authors suggest that it could be harbouring BYMV in the off-season and moving it to cultivated legume plants.

Table 23. Alternative hosts of key viruses detailed in this review

Virus	Alternate host species
PSbMV	Lentil *(Lens culinaris),* chickpea *(Cicer arietinum),* broad bean *(Vicia faba),* shepherd's purse, Medicago spp.*,* fenugreek, *Chenopodium spp.*
BLRV	Alfalfa (*Medicago sativa*), white clover (*Trifolium repens*), broad bean (*Vicia faba*), common bean (*Phaseolus vulgaris*), chickpea (*Cicer arietinum)*, cowpea (*Vigna unguiculata*) and lentil (*Lens culinaris*)
BYMV	Common bean (*Phaseolus vulgaris*), *Gladiolus sp., Freesia sp., Lupinus luteus, Robinia pseudoacacia, Trigonella foenum-graecum, Vicia sativa, Papaver somniferum , Arachis hypogaea, Crotalaria spectabilis,* Soybean (*Glycine max*), *Canna spp., Trifolium subterraneum, Eustoma russellianum*, broad bean (*Vicia faba*), alfalfa (*Medicago sativa*), *Trifolium spp.*

Detection and Identification of Viruses

AHDB Horticulture project FV453 highlighted the limitations in the number of reports, as well as commercially available methods of seed testing for both presence of virus and methods applied to support pea seed health and trade compliance. Visual assessment is not as accurate as diagnostic testing, often because of latent symptoms, the inability to distinguish between virus and nutritional deficiency symptoms, or it may simply just be too difficult to provide an accurate diagnosis in the field (Bekele *et al*., 2005; Bos, 1982; Coutts *et al*., 2008;

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Fox *et al*., 2017; Jain *et al*., 2013, Robert *et al*., 2000). Much of the work to date has centred on ELISA and tissue blot immunoassay (TBIA), with the International Seed Testing Association (ISTA) adopting a standardised and internationally validated method for PSbMV and PEBV due to the inherent risk of seed born virus diseases (ISTA, 2014). Further confirmation testing using methods based on viral nucleic acid and/or coat protein are necessary, but there is limited evidence of pea-specific molecular assays or advanced diagnostic techniques using NGS methods. The following section highlights physical, biochemical and nucleic acid characteristic features of the three key virus detailed in this review and studies which have employed relevant techniques to diagnostic or provide further insight.

Detection and Identification Methods Based on Biological Properties

Pea seed-borne mosaic virus (PSbMV)

Virions of PSbMV are filamentous, non-enveloped and usually flexuous with a clear modal length of 770 nm and 12 nm wide (Inouye, 1967; Hampton *et al*. 1981; Makkouk *et al*., 1993). The density of PSbMV in CsCl is recorded as 1.329 g cm^{-3}. Microscopy of PSbMV has shown that virions of PSbMV are found in the roots in cortical parenchyma. Inclusions are present in infected cells, resembling pinwheels which are unusual in shape; they aggregates in tonoplasts (Brunt et al. *et al*., 1996; Dallwitz, 1980; Dallwitz *et al*, 1993). PGRO currently offer a seed testing service for PSBMV based on ELISA, using a method adopted by ISTA (ISTA, 2014). Immunosorbent electron microscopy (ISEM) is also used to successfully diagnose plants infected with PSbMV and is more sensitive to detection than ELISA (Hamilton and Nichols, 1978). Wang *et al.,* 1993 studied twenty five UK varieties including Maro and Princess and showed all were susceptible to PSBMV, as detected by ELISA. Beck *et al*., 2018 used DAS-ELISA to detect PSbMV from tissue of 5 week olds plants of the field pea cv. Vegas. Tissue from the newest growth of PSbMV-positive plants was ground and inoculated onto 4-week-old cv. Ginny which were then re-tested with DAS-ELISA; virus-positive plants were subjected to RT-PCR using PSbMV-specific primers (Safarova *et al*., 2014).

Bean leaf roll virus (BLRV)

Leaf sap infected with BLRV is reported to contain few virions. Virions of BLRV are isometric and non-enveloped, ca. 27 nm in diameter and rounded in profile without a conspicuous capsomere arrangement. The density of BLRV in Cs_2SO_4 is 1.32 g cm^{-3}. Microscopy studies have shown that virions can be found in phloem parenchyma mainly in necrotic sieve-tube cells, with inclusions absent from infected cells. BLRV has been detected by ELISA in dry pea, lentil and chickpea with associated symptoms of leaf rolling, stunting, foliar chlorosis, and mosaic symptoms (Hampton 1983; Klein *et al*., 1991). Hajiyusef *et al*., 2017 describe the

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use of ELISA and TIBA for detection of BLRV in chickpea. Samples which proved serologically positive for BLRV were further confirmed by RT-PCR using BLRV specific primers. The most recent report on BLRV detection comes from (Agindotan *et al*., 2019) who tested fourteen plants of chickpea, dry pea and lentil for BLRV using a double antibody sandwich ELISA followed by RT-PCR using BLRV specific primers (Chomic *et al*., 2010). This is further discussed here.

Bean yellow mosaic virus (BYMV)

Virions of BYMV are filamentous, non-enveloped and usually flexuous; with a clear modal length of 750 nm and 12-15 nm wide (Bos *et al*., 1974; Moghal and Francki, 1981). Microscopy has shown that virions can be found in all parts of the host plant; inclusions are present in infected cells as crystals in the nucleus and cytoplasm, and pinwheels. Inclusion do not contain virions, although sometimes banded bodies are found in the cytoplasm that consist of virions. Plants with BYMV can be tested successfully by both ELISA with both polyclonal and monoclonal antibodies available for detecting BYMV in plant tissue (Werkmeister and Shukla, 1991; Ali, 2017) The most recent report by Wang *et al*., 2019 describes the successful use of a triple-antibody sandwich ELISA with a BYMV-specific antibody (DSMZ, Braunschweig, Germany) for detection of BYMV in nasturtium; this is further discussed here.

Detection and Identification Methods Based on Viral Nucleic Acid

Pea seed-borne mosaic virus (PSbMV)

PSbMV is a positive-sense, ssRNA virus (Ali and Randles, 1998; Beck *et al*., 2018). Virions of PSbMV contain 5.3% nucleic acid; 94% protein and 0% lipid. RT-PCR methods to test for PSbMV has long been established (Kohnen *et al*., 1992). Navrátil and Šafářová, 2019 obtained whole genome sequences of three Czech PSbMV isolates belonging to the P1 pathotype and causing different symptom intensity. The study detected for the first time natural recombination within PSbMV isolates of an important pathogen of leguminous plants.

(Cerna *et al*., 2017) present the results of a proteome-wide analysis of the response of peas to PSbMV infection. LC–MS profiling of a resistant and susceptible pea cultivar to PSbMV infection, detected >2300 proteins, 116 of which responded to PSbMV ten and/or twenty days post-inoculation. The work was further complemented by targeted analyses of free amino acids and selected small molecules, fatty acid profiling, and enzyme activity assays. These differentially abundant proteins are involved in a number of processes that have previously been reported in the plant-pathogen response; most interestingly, there were great similarities in the resistant and susceptible cultivars implying that even though no viral replication was

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detected in the PSbMV-resistant cultivar, it is still significantly affected by PSbMV inoculation at the proteomics level.

Bean leaf roll virus (BLRV)

The genome of BLRV is unipartite and consists of linear ssRNA with a total genome size of 6 kb. Virions of BLRV contain 37.8 % nucleic acid. Although polyclonal and monoclonal ELISA antibodies are available for BLRV detection (Makkouk and Kumari, 2009, Vemulapati *et al*., 2014), nucleic acid-based molecular approaches are favoured, (Figueira *et al*. 1997, Ortiz *et al*., 2005, Trucco *et al*., 2016). Currently available RT-PCR primers (Prill *et al*., 1990, Makkouk and Kumari, 2009) can detect BLRV presence in both aphid and plant tissues (Ortiz *et al*., 2005. Hajiyusef *et al*., 2017 employed RT-PCR to further confirm the presence of BLRV in samples of chickpea which proved serologically positive for the virus. Sequence comparison of the amplified products showed that the Iranian isolate in their work showed similar homology with isolates in South and North America and Europe. (Agindotan *et al*., 2019) used RT-PCR to test for BLRV in chickpea, dry pea and lentil, using BLRV specific primers developed by Chomic *et al*., 2010. One amplified BLRV partial coat protein (CP) product per crop variety was purified by ethanol precipitation, cloned in a pJet1.2 blunt vector and sequenced by Sanger's method using pJet1.2 forward and reverse primers. All the symptomatic chickpea, lentil, and dry pea leaf samples tested positive to BLRV by both ELISA and RT-PCR.

Bean yellow mosaic virus (BYMV)

Virions of BYMV contain 5% nucleic acid and 95% protein. The virion genome is monopartite consisting of positive-sense ssRNA with a total genome size of 10 kb (Makkouk *et al*. 2012). The coat protein of BYMV has been reported by Reddick and Barnett, 1983 to degrade if not purified rapidly. Primers have been developed for the detection of BYMV (Sharma *et al*., 2015), with PCR, one-step RT-PCR, real-time (rt)-RT-PCR and Immuno Capture (IC)-rt-RT-PCR all used for detection of BYMV in plant tissue (Duraisamy *et al*., 2011; Sharma *et al*., 2015).

Analysis of coat protein sequences from isolates of BYMV collected across four continents revealed seven distinct groups (Wylie *et al*., 2008). Kaur *et al*., 2017 report the complete genome sequence of five BYMV isolates that share 74.6- 98.9% (nucleotide) and 81.5-99.1% (amino acid) identity with globally available BYMV sequences. Phylogenetic analysis clustered them specifically into BYMV phylogenetic group-IV within the existing nine groups. Additional infectivity assays using in vitro RNA transcripts from two individual subgroups showed distinct biological differences between the isolates, supporting subdivision. Wang *et al*., 2019 tested nasturtium plants showing BYMV symptoms as confirmed using ELISA plus

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three non-infected plants with a universal potyvirus-specific RT-PCR with potyvirus nuclear inclusion body (NIb) primers (Zheng *et al*., 2010). All of the symptomatic nasturtium samples tested positive for potyvirus infection in the RT-PCR assay, with the non-symptomatic leaves testing negative. To identify the specific potyvirus involved, amplicons of the NIb region, previously generated by RT-PCR were sequenced. BLASTn analysis of the sequences showed that this virus shared 93% nucleotide identity with a BYMV isolate from Australia and BLASTx analysis showed it shared 93% amino acid identity with a polyprotein of BYMV isolate from Australia.

Novel technologies

No specific information was discovered on the use of robotics or aerial imagery for the detection of viruses in pea crops. A confidential project has recently started in peas and beans to investigate the use of remote sensing and advanced data analysis to improve crop production.

Modelling and decision support systems

Decision support systems for pea growers in the UK are centred around aphid monitoring networks provided by AHDB, Warwick University and Rothamsted Research and aphid predictions based on average winter temperatures. This is further discussed in the sections on potatoes, oilseed rape and carrots respectively. Congdon *et al*., 2017 investigated drivers of PSbMV epidemics in pea crops by collecting aphid occurrence and PSbMV epidemic data from twenty three data collection blocks over a six year period under a diverse range of conditions. The authors found that the magnitude of PSbMV spread prior to crop flowering is determined by PSbMV infection incidence in the seed sown pre-sowing rainfall which promotes vegetation growth driving early season-aphid populations. Following on from this work, Congdon *et al*., 2017b developed a model to forecast PSbMV incidence at a critical phase of the annual growing season to predict yield loss in field pea crops sown under Mediterranean-type conditions. The model uses pre-growing season rainfall to calculate an index of aphid abundance in early-August which, in combination with PSbMV infection level in seed sown, is used to forecast the incidence of virus in the crop.

Forecasts provided by the model allows sufficient time before sowing to implement control recommendations e.g. having seed tested, obtaining clean seed or a PSbMV-resistant cultivar, and implementation of cultural management strategies. The forecast also delivers location-specific recommendations to end-users via SMS alerts with links to web support for PSbMV management options. Though the system was developed for Mediterranean type growing environments, this approach would likely be suitable for use in other world regions, including the UK.

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Chemical and cultural management options.

The principles of virus management in peas has been extensively covered by Fox *et al*., 2017 in AHDB Horticulture project FV453. The section on potato virus management details the use of straw mulching, crop borders and mineral oil spraying for effective management of viruses and their associated vectors on seed and ware potatoes and the section on OSR discuss the suitability of biopesticides and physical barriers such as fleeces, nets, or insect traps for vector management. The efficacy of some management option may vary based on the mode of transmission, in persistent and non-persistent viruses. Moreover, regional agricultural practices and cropping systems need to be taken into consideration, e.g. planted pasture legumes and/ or cover crops may serve as overwintering reservoirs for both viruses and their insect vectors, thus contributing to virus spread and potential epidemics (Rashedetal *et al*., 2018).

Problematic weeds in legumes can be targeted in other crops in the rotation as there are significantly more active substances available for weed control in wheat and barley. Inter cropping can provide peas with a scaffolding that improves standing ability. In 2017 peas were grown with intercrops of spring oats, barley and oilseed rape, there was an indication that the intercrops supressed weed levels. Weed suppression in barley intercropped with peas was compared with peas or barley alone in five European countries (Italy, UK, Denmark, France, and Germany) (Corre-Hellou *et al*., 2011). Fat-hen (*Chenopodium album*) and charlock (*Sinapis arvensis*) were the two dominant weed species and their intensity and biomass were reduced in intercropped plots compared with plots in which peas were grown on their own or those that were kept fallow. *Chenopodium* spp. are a common alternate host for many common viruses, including PSbMV. For aphid transmitted viruses such as BLRV, infection may be prevented or reduced through timely application of aphicides, with applications related to aphid presence (see https://ahdb.org.uk/aphid-news and https://www.syngenta.co.uk/ahdb-pest-bulletin for further information).

For seed borne viruses such as PSbMV, reduction is more difficult to achieve with aphicides as the virus is non-persistent, therefore transmitted quickly by the pea aphid, black bean aphid and peach-potato aphid. It is also thought to be spread by migrating cereal aphids searching for a suitable host. Thus, use of virus-free, tested seed is the principal means to control infection.

Elsewhere in the world, Kaur *et al*., 2019 describe a method used to eliminate BYMV from an infected gladiolus crop in vitro using cormel explants which were subjected to thermotherapy (37°C for 30 days), chemotherapy (30 mg/L ribavirin for 30 days), and electrotherapy (30 mA for 20 min), either alone and in different combinations. The *in vitro* regenerated plants were

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free from BYMV infection when checked by RT-PCR using BYMV-specific primers. The combination of electro- and chemotherapies gave the best response as compared to other treatments, while electrotherapy (30 mA/20 min) proved to be the best for individual therapy production of BYMV-free gladiolus plants. This method has the potential to be transferred to other crop species as an effective virus management strategy for quality improvement of explants. Weed management for pulse growing regions of south-eastern Australia is discussed by Freeman and Aftab (2011) and can be adapted in other pulse growing regions as a component of an IPM approach. Roguing, though useful for small-scale farms and research plots, may help to reduce virus spread, however, is not a feasible practice in large-scale farming and could potentially stimulate aphid dispersal where large populations are present; a review by Makkouk and Kumari (2009) further discusses the use of this on farms in the United States. A review by Godfray *et al*., (2014), discusses the potential link between second-generation insecticides such as the neo-nicotinoids and bee mortality, especially in legumes that are frequently visited by pollinators; providing an essential reminder on the responsible and wise use of insecticides.

Breeding for aphid resistance and host resistance and/or tolerance

Planting resistant genotypes can be considered one of the most important components of IPM, particularly in vector-borne pathogen complexes. There are no virus resistant varieties on the 2019 PGRO Recommended List for combinable peas for cultivation in the UK (PGRO, 2019). Nonetheless, for viruses such as PSbMV, using highly tolerant varieties are an important part of disease management and resistance genes which are specific to pathovars of PSbMV can be found in the USA (Beck, 2018) and Australia (Congdon, 2016). Exploiting varietal resistance is an important part of PSbMV control, highlighted by Coutts *et al*., 2008 who reported stark differences in the resistance and susceptibility status of different pea genotypes. Several pea cultivars with resistance to PSbMV have been identified in the United States and several pea accessions from the Pisum Core Collection located at the USDA Western Regional Plant Introduction Station have been identified as resistant to all three pathotypes of PSbMV (Alconero *et al*., 1986). Resistance to PSbMV in pea is conferred by single recessive genes termed *sbm-1, sbm-2, sbm-3*, and *sbm-4* (Hagedorn and Gritton 1973, Provvidenti and Alconero 1988). In lentil, resistance to PSbMV is associated with the single recessive gene *sbv* (Haddad *et al*., 1978); however, current cultivars lack this gene and are susceptible to the virus. In peas, BLRV resistance and tolerance are controlled by the recessive genes *lr* and *lrv*, respectively (Makkouk *et al*., 2014). van Leur *et al*., 2013 successfully tested Australian pea varieties and breeding lines with resistance to BLRV in a series of experiments in Syria. Makkouk *et al*., 2002 screened 358 broad bean genotypes

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worldwide to detect sources of resistance to BLRV; 15 genotypes were identified (Makkouk *et al*., 2014).

Most pea varieties are resistant to BYMV since they have been bred to possess the single recessive gene *mo* which confers resistance to this virus (Yen and Fry, 1956). An additional single recessive resistant gene in pea, *Pmv*, also has been shown to confer resistance to BYMV (Provvidenti, 1990). Resistance in broad bean to BYMV has been identified with accession 2N138 showing immunity to two different BYMV strains (Gadh and Bernie,r 1984) and eight genotypes immune to a Syrian strain of BYMV (Makkouk and Kumari 1995). In addition, two recessive resistant genes *bym-1* and *bym-2* have been identified in broad bean conferring resistance to BYMV (Rohloff and Stulpnagel, 1984, Schmidt *et al*., 1985).

A 2017 patent pending describes the breeding of a pea variety in the USA with “En” allele for resistance to PEMV (Plouy, 2017). Rana *et al*., 2014 report the evaluation of germplasm of kidney bean (4274 accessions), pea (701), rice bean (458), adzuki bean (116), horse gram (118) and cowpea (228) for agronomic, disease and quality traits. Trait specific sets of germplasm have been developed for, including resistance to *bean common mosaic virus* (BCMV) in kidney bean.

Schafleitner *et al*., 2014 report on the development of methods for next generation phenotyping in mungbean. The authors used restriction site associated DNA (RAD) sequencing and genotyping by sequencing (GBS) methods on mungbean breeding parents and mapping populations to produce a critical number of polymorphic SNP markers for molecular breeding purposes. Mapping of resistance genes against *Mungbean yellow mosaic virus* (MYMV) is ongoing in this population. Boersma *et al*., 2014 used marker-assisted selection in an effort to transfer common bacterial blight resistance to dry bean cultivars and pyramid resistance to multiple diseases. The developed breeding populations were screened in nurseries for bacterial blight and in growth chambers for resistance to anthracnose and BCMV. Navy bean, pinto and black bean lines with good resistance to BCMV were produced. *Faba bean necrotic yellows virus* (FBNYV) is persistently transmitted by various aphid species, and most efficiently by *A. pisum* and *A. craccivora*, with epidemics reported in several countries in West Asia and North Africa. Around 1000 faba bean accessions with a wide genetic background were evaluated over the course of 10 years for their reaction to FBNYV using artificial inoculation with *A. pisum* under field conditions (Kumari *et al*., 2014). No complete resistance was found, however five new breeding lines with resistance to FBNYV were developed.

Genetic modification and CRISPR technology

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There is limited information on the development of transgenic peas showing resistance to viruses in the literature. Advances in developing transgenic pulse crops have primarily been limited to laboratory trials and have not been commercialized at the large scale (Eapen, 2008), with the exception of the release of a RNAi-mediated resistant pinto bean to BGMV in Brazil (Bonfim *et al*., 2007, Tollefson, 2011). In FV453, Fox *et al*., 2017 discuss a number of studies which were conducted in the late 1990's and early 2000's (Chowrira *et al*., 1998, Jones *et al*., 1998, Timmerman-Vaughan *et al*., 2001). Genomic resources, such as bacterial artificial chromosome (BAC) libraries, are available for various pulse crops including peas, chickpea, and beans, enabling the development of molecular markers for marker-assisted selection towards improved yield and resistance to a wide range of pathogens (see Yu, 2012 and Meziadi *et al*. 2017 for reviews). The BAC cloning is a cost-effective method to maintain and manipulate large sequences of DNA (Yu, 2012).

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Virus management in vegetable Brassicas

As with OSR, there are several viruses which affect vegetable Brassica crops-members of the *B. oleracea* family-in the UK with *turnip yellows virus* (TuYV) having the greatest impact in terms of the proportion of crops affected and yield reductions. In 2016, Over 27,300 ha of vegetable Brassicas were grown in the U.K. amounting to a total home production market value of £265 million, with a hectare of winter cabbage worth £12,119 (Defra Horticultural Statistics, 2018). As with OSR, the loss of neonicotinoid seed treatments and increasing issues of insecticide resistance has reduced pest control options, substantially increasing the risk of yield reductions and crop failure. However, unlike OSR, less is known about the effect of viruses on vegetable Brassicas, other than research available on cabbage. It is essential that these crops be kept disease free, particularly for long season crops such as overwintered cauliflower and Brussels sprouts where they are exposed to infection for a longer period of time and there is much greater opportunity for the virus to affect tonnage and quality because of the long growing period.

This review follows on from, and supplements the research conducted by Walsh *et al*., 2011 as part of the AHDB Horticulture funded project FV365, '' The incidence of *Turnip yellows virus* (TuYV) in overwintered cauliflower and Brussels sprout and the effect of the virus on yield, quality and storage. The section on virus management in OSR details information on the key viruses of Brassica crops, focusing on TuYV in OSR along with detection methods for TuYV, available modelling and genetic tools, crop management and advances in breeding for resistance. Thus the aim of the present review will be to supplement that presented in for OSR with up to date research specific to vegetable Brassica crops.

Increased diversity of current viruses

There are four potential viruses of vegetable Brassicas in the UK; TuYV, *cauliflower mosaic virus* (CaMV), *turnip mosaic virus* (TuMV) and *turnip yellow mosaic virus* (TYMV). The section on virus management in OSR details further information on these viruses, including genus and mode of transmission of each. In FV365, Walsh *et al*., 2011 reported an incidence of TuYV of up to 60% in cauliflower crops and 55% in Brussels sprout crops. Controlled experiments in gauzehouses showed that TuYV induced very little leaf symptoms in Brussels sprout or cauliflower plants, however growing infected Brussels sprout plants next to uninfected plants revealed clearly that TuYV infection had stunted plant growth in all seven varieties tested. TuYV reduced the marketable yield of these Brussels sprout varieties by up to 65% with even the highest yielding Brussels sprout variety having its marketable yield reduced 30% by TuYV. TuYV also significantly reduced the shelf life of two of the seven sprout varieties tested.

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TuYV is the primary cause of tipburn in cabbage. TuYV also reduces growth in the field and this can result in up to 25% reduction in harvest tonnage (Walsh *et al*., 2011). Cigar burn symptoms are caused by the aphid-borne TuMV. Severe TuMV infection of the cabbage leaves can occasionally be seen in the growing crop, prior to harvest, as black rings and necrotic spots on the older leaves. In addition to leaf quality problems, yield losses of up to 20% head weight can also be experienced when virus infection has occurred. Symptoms of both tipburn and cigar burn are exacerbated when CaMV is present in combination with either TuYV or TuMV. If the cabbage has a mixed infection of CaMV and TuMV, the internal disorders are more severe and losses can be as high as 65%. Broccoli necrotic yellows (BNYV), a Cytorhabdovirus is also present in the UK and has been recorded (Walsh, J. Pers. Comm.).

New emerging viruses

Unlike OSR, vegetable Brassica crops are susceptible to numerous other viruses; Plant Virus Online details 15 viruses which can infect crops of Brussels sprouts, cabbage, cauliflower and kale however these are not present in, or do not have the potential to infect vegetable Brassicas grown in the U.K.

Table 24. Viruses which are known to infect crops of Brussels sprouts (*Brassica oleracea* var. *gemmifera*), cabbage (*B. oleracea* and *B. oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botrytis*) and kale (*B. oleracea*) but have not yet been identified in crops in the United Kingdom.

Virus	**Acronym**	**Genus**	**Susceptible**
Arabis mosaic	ArMV	Nepovirus	Cauliflower
Arracacha A	AVA	Nepovirus	Cabbage (var. capitata)
Broccoli necrotic yellows	BNYV	Cytorhabdovirus	Brussels sprouts, cabbage, kale
Cassia mild mosaic	CasMMV	Carlavirus	Cabbage (var. capitata)
Cole latent	CoLV	Carlavirus	Cabbage, Cabbage (var. capitata), cauliflower, kale
Radish mosaic	RaMV	Comovirus	Cabbage, kale
Erysimum latent	ErLV	Tymovirus	Cauliflower
Plantago 4	PlV-4	Caulimovirus	Cauliflower
Pea seed borne mosaic	PSbMV	Potyvirus	Brussels sprouts
Pepper veinal mottle	PVMV	Potyvirus	Cabbage (var. capitata)
Primula mosaic	PrMV	Potyvirus	Cauliflower
Ribgrass mosaic	RMV	Tobamovirus	Cauliflower
Strawberry latent ringspot	SLRSV	Nepovirus	Cauliflower
Tomato spotted wilt	TSWV	Tospovirus	Cauliflower
Turnip crinkle	TCV	Carmovirus	Cauliflower

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Vectors and alternate hosts of key viruses

The previous section on OSR provides information on TuYV and its associated vectors and alternate weed hosts.

Detection and Identification Methods Based on Biological Properties

The previous section on OSR provides more information on the biological properties of TuYV and detection methods which are used based on these properties. TuYV can be detected in infected cabbage heads during the growing season with ELISA based methods. However, only 50% of virus-infected heads go on to develop symptoms. This is not considered a reliable enough indication on which to base store management decisions (Walsh *et al*., 2004). Monitoring should therefore be based on cutting open heads both pre-harvest and in store. If symptoms start to appear, growers should be aware that these would get worse with time, so should consider shortening the period the heads are in store.

Detection and Identification Methods Based on Viral Nucleic Acid

(Congdon *et al*., 2019) describe the development of a new TuYV diagnostic protocol for OSR using a novel crude extraction method and RT-LAMP assay performed on a portable instrument. Inexpensive and rapid TuYV detection can be performed on suspected viruliferous aphids caught in traps as well as potential host plants. This protocol is detailed further under Virus management in OSR. Although developed for OSR, it is feasible that the methodology could be transferred to vegetable Brassica crops also.

Novel technologies

No specific information was discovered on the use of robotics or aerial imagery for the detection of viruses in vegetable Brassica crops.

Modelling and decision support systems

Decision support systems for Brassica growers in the UK are centred around aphid monitoring networks provided by AHDB, Warwick University and Rothamsted Research and aphid predictions based on average winter temperatures. Mean temperatures in January and February is the dominant factor affecting aphid migration phenologies for *M. persicae*, who pass the winter in the active stages (as opposed to eggs). A 1°C increase in average winter temperature advances aphid migrations by 4-19 days depending on the species (Zhou *et al*., 1995). *B. brassicae* overwinters mainly in the active stages; it flies later and is more difficult to predict, thus December temperatures are also taken into account for this aphid. (Congdon *et al*., 2019) demonstrated using epidemiological modelling of the TuYV rapeseed pathosystem in the south-west Australian grain belt that high levels of rainfall prior to sowing

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(late-summer to early-autumn) drive establishment of a large infection reservoir and aphid vector population. This model is detailed further in under virus management in OSR. Again; although developed for OSR, it is feasible that the methodology could be transferred to vegetable Brassica crops also.

Chemical and cultural management options.

The principles of virus management as detailed by Fox *et al*., 2017 in FV453 are applicable across multiple crops and cropping scenarios. The section on potato virus management provides extensive detail on the use of straw mulching, crop borders, intercropping and mineral oil spraying for effective management of viruses and their associated vectors on seed and ware potatoes. The section on OSR discuss the suitability of biopesticides and physical barriers such as fleeces, nets, or insect traps for vector management.

Cultural control

Most cabbage aphid infestations develop from colonies that overwinter on old brassica crops and autumn sown OSR. Plough in or otherwise destroy old crop residues to help reduce aphid populations. Cabbage crops intended for short, medium and long term storage should not be grown in fields close to overwintering brassicas, especially OSR, as these can be important sources of viruses, through aphid transmission. Selecting vegetable brassicas with high chlorophyll and water content has been shown to reduce *B. brassicae* abundance, while thin leaves with a low protein content will reduce honeydew damage to prevent unmarketable vegetables (Munthali and Tshegofatso, 2014). Simon *et al*., 2014 showed that in Mediterranean France, a permanent mesh net covering a cabbage crop significantly reduced *B. brassicae* populations, but had no effects on *M. persicae*. This is probably explained by the netting size (0.73 mm and 1.6 mm) and the more globular and larger size (>3 mm) of *B. brassicae* compared to *M. persicae* which is <1.5 mm in length. In areas where *B. brassicae* is a major pest, netting over OSR could be adopted, however, considerable labour would be involved. Additionally, no research has explored the potential exclusion of smaller aphids using finer nets, and whether these would affect the microclimate.

Monitoring and avoidance

Field inspections should be made for the symptoms of viruses by cutting open and inspecting cabbage heads. Testing cabbage for TuYV in the field pre-storage by ELISA does not accurately estimate level of tipburn symptoms in the crop.

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Biological control

Natural enemies of *B. brassicae* include parasitic wasps, ladybirds, hoverflies, lacewings, predatory flies, spiders, and insect-pathogenic fungi. Providing diverse habitats to harbour predator and parasitoid communities may help to control *B. brassicae*, however these predator-prey assemblages may not be effective in preventing virus transmission however, as even low aphid densities can result in virus transmission. Chaplin-Kramer *et al*., 2013 investigated the influence of natural habitats on natural enemy populations and its effect on *B. brassicae* populations within organic broccoli in North America. By analysing weekly insect samples over three years, the authors found that hoverfly diversity was positively correlated with natural habitat at all spatial scales but was stronger and only significant at smaller scales. As all of the sites were of organic status, Chaplin-Kramer *et al*., 2013 were confident that the positive response of hoverfly populations was a result of natural habitat abundance, as opposed to pesticide intensity, which has been considered a stronger driving force of natural enemy abundance by some (Hendrick *et al*., 2007; Jonsson *et al*., 2012). Hoverflies are dependent upon floral resources during their adult life stage (Kos *et al*., 2012. Van Rijn *et al*., 2013). Van Rijn *et al*. 2013 found that honeydew consumption by hoverflies enhances adult hoverfly survival in the presence and absence of abundant floral resources, with one fertile female hoverfly producing enough offspring that were capable of supressing the growth of a cabbage aphid colony, originally consisting of 30 individuals. On the contrary, parasitoids often perform significantly worse when utilizing aphid honeydew (Wäckers *et al*., 2008). Prasad and Kabaluk, 2009 suggested that for effective biological control of aphids, hoverflies should oviposit their predatory offspring in the crop when aphid numbers are low.

To investigate the effect of manipulated density on the wolf spider (*Pardosa astrigera*) in small cabbage (*Brassica oleracea* L. var. *capitata*) plots, Suenaga and Hamamura, 2015 found that increased spider activity density could be ascribed to the application of straw mulch which may have provided refugia for the spiders. Application of straw mulch caused the spiders to disperse reducing spider-spider interaction rates. Spiders are known to have cannibalistic tendencies, and therefore, refugia provision may reduce cannibalistic interactions (Halaj *et al*., 2000; Langellotto and Denno, 2006; Rickers and Scheu, 2005). Although straw mulch may not be a feasible option within large-scale UK agriculture, it does demonstrate that by diversifying the system, intraguild predation can be reduced, allowing for stable predator populations. Weed cover can also act as a refuge; where possible, conservation headlands could be adopted to improve predator refuge in field perimeters.

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Chemical control

Current aphid control measures are based around a number of active substances including flonicamid, thiacloprid, Cyantraniliprole, spirotetramat, synthetic pyrethroids and chlopyrifos under protection. Preventing early infection of TuYV whilst plants are small is crucial for both yield and to limit storage losses. Loss of Cruiser 70WS (thiamethoxam) and Plenum WG (pymetrozine) has caused concerns with regard to TuYV in long growth cycle Brassica crops such as storage cabbage and Brussels sprouts. Use of a seed treatment is the best way of controlling virus spread early on in the crops life; it is these early infections that give significant yield loss as well as internal issues in cabbage. Shorter season crops, including broccoli, however, are still frequently grown without a seed treatment, and frequently require a full foliar programme. However, foliar sprays simply don't work as well at combatting spread (Anonymous, 2018). Cruiser 70WS was reported to provide eight to ten weeks control of aphids, which could in itself prove sufficient, but typically needed to be supplemented with two or three foliar insecticides, according to the crop and the seasonal pressures. Plenum WG was very effective at reducing virus transmission by stopping aphids feeding quickly and, in the case of persistent viruses such as TuYV, completely protect plants from virus infection. Whilst aphids are actively feeding in warmer conditions, an insecticide with ingestion take up is advised followed by a switch to contact products when conditions cool down in the autumn if aphids are still active.

Bayer have recently registered Sivanto (flupyradifurone) in the Netherlands. This is a butenolide – a new class of insecticides, which acts on the central nervous system of insects. Although not a neonicotinoid, it could be positioned similarly in terms of rapid efficacy, quick knock-down and positive effects on virus transfer. This active has possibilities to be available in vegetable. It is likely to be limited to only 1 x application per year per crop Requiem a biological-type insecticide based on the terpenoid blend QRD460 is also in the registration process in the Netherlands, initially for protected crop uses, but could be looked at on outdoor crops too. With a zero residue profile, it is mainly targeting small sucking pests, so includes some aphid species, some mites and some thrips. It has a relatively rapid knock-down effect, but does not persist on the leaf surface (Lacey, T., Pers. Comm.). Syngenta acquired DevGen in 2013 to develop RNAi as a sprayable crop protection product to control insects; this could be a future tool for virus management, the company are also developing a biostimulant that helps elevate the effect of viruses if infection occurs. Both products are currently confidential (Newbert, M. Pers. Comm.).

Elsewhere in the world; in Ghana, Ngosong (2017) investigated the effects of six different pest management strategies on key insect pests of cabbage; *B. brassicae*, *Plutella xylostella*,

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and *Hellula undalis*. Plots were treated with either an aqueous neem seed extract, Bypel 1, shallots planted 14 days or 7 days prior to cabbage transplanting, and shallots planted with cabbage on the same day combined with a short duration of neem spray. While treatment with Bypel 1 had the least number of *P. xylostella,* and *H. undalis*, shallot planted the same time with cabbage, then sprayed with a short duration of neem had the lowest overall aphid score. Shallot plots planted 14 days before transplanting cabbage had the highest numbers of natural enemies. Yield and marketability of cabbages was also increased for plots treated with Bypel 1, sole neem, and shallot with short duration neem.

Breeding for aphid resistance and host resistance and/or tolerance

The best control option for TuYV in vegetable Brassicas is natural plant resistance to the virus. It is possible to select cabbage varieties with some known resistance to the major storage disorders and virus diseases. Guidance on varietal resistance should be sought from the various seed companies. Past (NIAB) Descriptive Lists have rated winter white cabbage varieties for resistance to TuMV and CaMV. However, there are no independent trials data available for the full spectrum of current varieties used in the industry. Walsh *et al*., 2011 found that the highest marketable yield achieved for virus-infected Brussels sprout plants was from the variety Speedia which produced double the yield of virus-infected plants of two other varieties. This results show that in years with high aphid activity and a lot of TuYV infection, growers could double their profits by growing the least affected Brussels sprout variety rather than the most susceptible ones. Jahan *et al*., (2013) have also demonstrated the effect of cultivar selection on the development, reproduction, and longevity of the cabbage aphid in cauliflower plants.

Genetic modification and CRISPR technology

No reports to date have detailed the introduction of virus and/or insect tolerance into vegetable Brassicas using GM or gene editing approaches. However, these techniques are being developed for this crop group. Large differences in transformation efficiency among families, species and cultivars of the Brassicaceae have been reported (Klocke *et al*., 2010). Transformation is usually achieved by inoculation of seedling explants with Agrobacterium or direct gene transfer using protoplasts followed by regeneration through organogenesis, however this is strongly dependent on the genotype and various other factors. *B. rapa* genotypes are more difficult to transform than *B. oleracea* ones, with cabbage (*B. oleracea* var. capitata) the most difficult type within the latter. This is due to a strong genetic component with significant additive effects both for *A. tumefaciens* susceptibility and regeneration ability from cotyledon stage explants as well as from leaf protoplasts (Sparrow *et al*., 2004).

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In *B. oleracea*, CRISPR/Cas9 has been used to induce indel mutations in two regions of the BolC.GA4.a gene which is involved in gibberellin biosynthesis (Lawrenson *et al*., 2015). Regenerated plants showed a range of mutations in the target gene with two plants exhibiting the expected dwarf phenotype and alterations in pod valve margins.

Murovec *et al*., 2018 developed a DNA-free protocol for site-directed mutagenesis of *B. oleracea* 2018 by introducing ribonucleoprotein complexes (RNPs) into their protoplasts with PEG 4000. RNP transfection into *B. oleracea* protoplasts was achieved and generated efficient indel induction of two endogenous genes. This study paves the way for regeneration of precisely mutated Brassica plants without the use of transgenesis. Ma *et al*., 2019 have shown that CRISPR/Cas9 can be used to efficiently mutate genes of interest in cabbage. The three genes targeted were involved in coloration, self-incompatibility, and pollen development respectively.

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Virus management in cucurbits

Cucurbitaceae (Cucurbits) are a large plant family which contain food plants grown in the UK such as *Cucurbita* (squash, pumpkin and courgette) and *Cucumis* (cucumber) (Kumar, 2016). Although grown over a relatively small area in the United Kingdom (mostly in Cornwall, Cambridgeshire, Worcestershire, and Sussex), courgette (*Cucurbita pepo* L.) is a high-value crop (~£8,000 per ha) with production in the United Kingdom estimated to be worth £6.7 million to the UK economy by Knapp and Osborne (2017).

Current viruses present in the UK

More than seventy different viruses have been reported to cause cucurbit diseases and many are responsible for economic losses in the quality and quantity of cucurbit crops (Ali *et al*., 2012; Lecoq and Desbiez, 2012; Lecoq and Katis, 2014). Table 25 lists five of these viruses known to exist currently in the UK and infect courgette. This review will focus specifically on four key viruses which are the principle targets affecting courgette crops in the UK; namely *Cucurbit yellow stunting disorder virus* (CYSDV), *Cucumber Mosaic Virus* (CMV), *Watermelon mosaic virus* (WMV) and *Moroccan watermelon mosaic virus* (MWMV)

Table 25. Summary of viruses reported on courgette in the United Kingdom, together with, where available, information on their mode of transmission and their vector. Viruses highlighted in bold italics are further discussed in the present Section.

Virus	Acronym	Genus	Mode of transmission	Vector
Cucurbit yellow stunting disorder virus	CYSDV	Crinivirus	Non persistent, aphid transmitted	*B. tabaci.*
Cucumber Mosaic Virus	CMV	Cucumovirus	Non persistent, aphid transmitted	*Acyrthosiphon pisum, Aphis craccivora, M. persicae*
Watermelon mosaic virus	WMV	Potyvirus	Non persistent; aphid transmitted	*M. persicae, A. solani, A. craccivora, Macrosiphum euphorbia*
Moroccan watermelon mosaic virus	MWMV	Potyvirus	Non persistent, aphid transmitted	*A. gossypii, M. persicae, A. craccivora*
Zucchini Yellow Mosaic Virus	ZYMV	Potyvirus	Non persistent, aphid transmitted	*Aphis gossypii, M. persicae, A. craccivora*

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Cucurbit yellow stunting disorder virus (CYSDV)

CYSDV, a Crinivirus in the family Closteroviridae was first detected in Spain in 1992 and has become a prevalent virus in cucurbit crops of South Eastern regions of Europe (Abou-Jawdah *et al*., 2000; Desbiez *et al*., 2000; Kao *et al*., 2000, Louro *et al*., 2000) CYSDV induces interveinal chlorotic spots in mature leaves of its host which enlarge and may eventually coalesce, resulting in the yellowing of the entire leaf except for the veins which remain green. In some instances, the leaves will sometimes roll up and become brittle. In attempt to save itself, the infected plant often drops its older leaves. Without enough leaves, the plant's strength dwindles and it cannot support or nourish its fruit. As a result, fruits are smaller and don't ship or store as well (McGinley, 2010).

Cucumber Mosaic Virus (CMV)

CMV, a Cucumovirus in the family Bromoviridae was first discovered in cucumber by Price (1934) in the USA. There are numerous strains of CMV; the better known include: A-CMV, E-CMV, L-CMV, N-CMV, P-CMV, Z-CMV and WAI/WAII (Francki, 1980; Habili, 1987). CMV is one of the most common plant viruses and causes a wide range of symptoms, especially yellow mottling, distortion and stunting. CMV caused symptoms on foliage known as the "shoestring" effect where young leaves appear narrow and the entire plant is stunted. CMV can cause fruits to turn pale and bumpy. The leaves of these plants turn mosaic and their rugosity is often changed, making leaves wrinkled and misshapen (Doolittle, 1920; Francki *et al*., 1979, Palukaitis *et al*., 1992).

Watermelon mosaic virus (WMV)

WMV was first reported in *Citrullus lanatus* by Webb (1965). Symptoms manifest as chlorosis of leaf veins with leaves developing a green mosaic and becoming deformed and blistered. Early plant infection often leads to severely distorted, discoloured fruit. When virus infection occurs after fruit set, generally fruit development is normal. WMV isolates are classified as WMV-1 and WMV-2 (Purcifull and Hiebert, 1979; Yeh *et al*., 1984). Isolates that infect only plants of the Cucurbitaceae family are designated WMV-2, while those that can infect outside this family are designated WMV-1. Purcifull and Heibert (1979) also a third isolate that did not react with antisera of either WMV-1 or WMV-2; this was classed as MWMV.

Moroccan watermelon mosaic virus (MWMV)

MWMV, a Potyvirus from the family Potyviridae was first reported in *C. lanatus* by Fischer and Lockhart (1974). It was detected for the first time in UK cucurbit crops in 2018. MWMV causes very severe mosaic and deformation of leaves and fruits. Systemic infection occurs as necrotic leaf spots, which is then followed by complete plant collapse. MWMV is almost

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exclusively restricted to cucurbits, whereas WMV has the widest host range among the potyviruses (Gilbert-Albertini *et al*., 1995; Kabelka and Grumet, 1997; Provvidenti, 1985; Wai and Grumet, 1995).

New emerging viruses

Table 26 lists the current viruses known to infect courgette according to Plant Virus Online (Brunt et al., 1996) but to the authors' knowledge have not yet been detected from courgette crops in the United Kingdom.

Table 26. Potential emerging viruses

Virus	**Acronym**	**Genus**
Squash leaf curl virus	SLCV	Bigeminivirus
Zucchini yellow mosaic virus	ZYMV	Potyvirus
Eggplant mottled crinkle virus	EMCV	Tombusvirus
Beet curly top virus	BCTV	Hybrigeminivirus
Beet western yellow virus	BWYV	Luteovirus
Lettuce infectious yellows virus	LIYV	Closterovirus
Arabis mosaic virus	AMV	Nepovirus
Lisianthus necrosis virus	LNV	*Necrovirus*
Squash leaf curl virus	SLCV	Bigeminivirus
Melothria mottle virus	Unknown	Potyvirus
Papaya ringspot virus	PRSV	Potyvirus
Peanut stunt virus	PSV	Cucumovirus
Prune dwarf virus	PDV	Ilarvirus
Prunus necrotic ringspot virus	PNRSV	Ilarvirus
Squash mosaic virus	SqMV	Comovirus
Tobacco ringspot virus	TRSV	Nepovirus
Tobacco streak virus	TSV	Ilarvirus
Trichosanthes mottle virus	TrMV	Potyvirus

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Vectors of key viruses

Cucurbit yellow stunting disorder virus (CYSDV)

CYSDV is transmitted by *B. tabaci*, in a semi-persistent manner, with the virus remaining virulent within the vector for up to nine days (Célix *et al*., 1996). All biotypes of *B. tabaci* can transmit the virus efficiently (Dinsdale *et al*., 2010; De Barro *et al*., 2011). Whitefly transmission allows for virus spread over short distances while spread over long distances is facilitated through the movement of infected plants, especially cucurbit transplants. Because it can take 3 to 4 weeks for disease symptoms to become detectable, infected symptomless plants can be transported unknowingly. (Célix *et al*., 1996) report that CYSDV can be acquired by whiteflies from infected melon plants with feeding periods as short as two hours, but acquisition periods of at least 18 hours resulted in transmission to greater than 80% of test plants. Transmission in the field is possible with low whitefly populations but as populations increase, transmission rates to cucurbit crops usually increase as well (Célix *et al*., 1996). CYSDV is neither seed borne nor mechanically transmitted (Tzanetakis *et al*., 2013).

Cucumber Mosaic Virus (CMV)

CMV has a wide host range, infecting more than 1,200 plant species (Chabbouh and Cherif, 1990). CMV is transmitted by more than 60 insect species, including *A. pisum*, *Aphis craccivora* and *M. persicae* in a non-persistent manner. The virus can be transmitted by mechanical inoculation, by seed in ca. 19 species to variable extents and by the parasitic weeds, Cuscuta spp. (Francki *et al*., 1979). CMV is not a very stable virus; when it is transmitted by aphids, this virus has an acquisition period of five to ten seconds and an inoculation period of about a minute. Nevertheless, after two minutes, the probability of inoculation largely decreases, and within two hours it is practically impossible to transmit it (Agrios, 1978, Francki *et al*., 1979).

Watermelon mosaic virus (WMV)

WMV is transmitted by many insect vectors including *M. persicae*, *A. solani*, *A. craccivora* and *M. euphorbiae* (Karl and Schmelzer, 1971) in a non-persistent manner. The virus can be transmitted by mechanical inoculation and is not transmitted by seed.

Moroccan watermelon mosaic virus (MWMV)

As with WMV, is transmitted by many insect vectors including *M. persicae*, *A. solani*, *A. craccivora* and *M. euphorbiae*. Recent experience in the UK has suggested that the virus may also be whitefly transmitted, but this is yet to be confirmed and would require a transmission test to be performed.

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Table 27. Vectors of the key viruses detailed in this review; CYSDV, CMV, WMV and MWMV

Virus	Vector species
CYSDV	*B. tabaci*
CMV	*A. pisum, A. craccivora, M. persicae*
WMV	*M. persicae, A. solani, A. craccivora, M. euphorbia*
MWMV	*M. persicae, A. solani, A. craccivora, M. euphorbia*

Alternate hosts of key viruses

Cucurbit yellow stunting disorder virus (CYSDV)

CYSDV has a relatively narrow host range the major hosts are: *C. lanatus*, *Cucumis melo* L., *C. sativus* L. and *C. pepo* (Louro *et al*., 2000). Wintermantel *et al*., 2009 demonstrated that lettuce, snap bean, alkali mallow, Wright's groundcherry, and buffalo gourd (*Cucurbita foetidissima*) could serve as virus reservoir hosts for transmission of CYSDV to melon and other cucurbits.

Cucumber Mosaic Virus (CMV)

CMV has a wide host range, infecting more than 1,200 plant species (Chabbouh and Cherif, 1990). CMV can overwinter in perennial plants and weeds, as it can survive the winter in the roots of the plant and move to the aerial parts in spring, where it can be transmitted by aphids to other plants (Francki *et al*., 1979).

Watermelon mosaic virus (WMV)

WMV presents a broader host range than most other potyviruses; experimentally WMV has been shown to infect more than 170 plant species from 27 different families (Shukla *et al*., 1994).

Moroccan watermelon mosaic virus (MWMV)

Plant Virus Online lists *C. lanatus, C. melo, C. sativus, C. pepo* and *M. parviflora* as susceptible hosts to MWMV (Brunt et al., 1996).

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Table 28. Alternate hosts of the key viruses detailed in this review.

Virus	Alternate host species
CYSDV	*C. lanatus*, *Cucumis melo* L., *C. sativus* L.*C. pepo*, Lettuce, snap bean, alkali mallow, Wright's groundcherry, buffalo gourd (*Cucurbita foetidissima*)
CMV	*Abelmoschus esculentus, Amaranthus spp., Antirrhinum majus, Apium graveolens, Atriplex hortensis, Beta vulgaris, Brassica spp., Calendula officinalis, Capsella bursa-pastoris, Capsicum spp., Catharanthus roseus, Cheiranthus cheiri, Chenopodium album, Chenopodium spp., Chrysanthemum morifolium, Cicer arietinum , Citrullus lanatus, Coriandrum sativum, Crotalaria spectabilis, Cucumis spp., Cucurbita spp., Datura spp., Emilia sagittata Fagopyrum esculentum, Glycine max, Gomphrena globose Gossypium hirsutum, Helianthus annuus, Hyoscyamus niger, Ipomoea nil, Lens culinaris, Lotus corniculatus, Lupinus spp., Lycopersicon spp., Matthiola incana, Medicago sativa, Melilotus albus, Momordica balsamina, Nicotiana spp. Phaseolus spp., Physalis floridana, Physalis peruviana, Phytolacca Americana, Pisum sativum, Raphanus sativus, Rumex acetosa, Senecio vulgaris, Solanum spp., Sonchus oleraceus, Spinacia oleracea, Tetragonia tetragonioides, Trifolium spp., Tropaeolum majus, Verbesina encelioides, Vicia spp., Vigna spp.,*
WMV	*Abelmoschus esculentus, Amaranthus caudatus, Astragalus sinicus Atriplex hortensis, Capsella bursa-pastoris, Cassia occidentalis Catharanthus roseus, Celosia argentea, Chenopodium spp., Cichorium endive, Citrullus lanatus, Coriandrum sativum, Crotalaria spectabilis, Cucumis spp., Cyamopsis tetragonoloba, Datura stramonium, Euphorbia marginata, Glycine max, Gomphrena globose, Lavatera trimestris Luffa acutangula, Lupinus spp., Macroptilium lathyroides, Macrotyloma uniflorum, Medicago spp., Melilotus albus, Melilotus officinalis, Nicotiana spp., Phaseolus vulgaris, Pisum sativum, Senecio vulgaris, Sesamum indicum, Sonchus oleraceus, Spinacia oleracea, Tetragonia tetragonioides, Torenia fournieri, Trifolium spp., Valeriana officinalis, Vicia spp., Vigna spp., Zinnia elegans*
MWMV	*Citrullus lanatus, Cucumis melo, Cucumis sativus, Cucurbita pepo, Malva parviflora*

Detection and Identification Methods Based on Biological Properties

Cucurbit yellow stunting disorder virus (CYSDV)

Leaf sap containing CYSDV can contain a variable number of virions. Virions present are isometric and non-enveloped, ca. 750-800 nm in length, 29 nm in diameter and rounded in profile without a conspicuous capsomere arrangement (Aguilar *et al*., 2006). The density of CYSDV is 1.367 g cm^{-3} in CsCl. CYSDV can be tested for with a range of different immunoassays; Jawdah *et al*., 2008 used tissue blot immunoassay (TBIA), dot blot immunoassay (DBIA) and ELISA to assess the movement of CYSDV in susceptible and tolerant cucumber germplasms. Keshavarz *et al*., 2013 screened 336 different cucurbit samples for the presence of CYSDV by indirect-ELISA (Converse and Martin, 1990) using a

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CYSDV specific polyclonal antibody (Keshavarz, 2003) and goat anti-rabbit alkaline phosphatase conjugate.

Cucumber Mosaic Virus (CMV)

Leaf sap containing CMV can have a variable amount of virions. Virions present are isometric, non-enveloped, ca 29 nm in diameter and rounded in profile; without a conspicuous capsomere arrangement. The density of CMV is 1.367 g cm^{-3} in CsCl (after fixation with formaldehyde). ELISA has been available for detection of CMV since 1978 (Gera *et al*., 1978).

Watermelon mosaic virus (WMV)

Leaf sap containing WMV contains many virions. Virions are filamentous, non-enveloped; usually flexuous; with a clear modal length of 760-800 nm and 12 nm wide. The density is 1.32 g cm^{-3} in CsCl. Antisera to purified preparations of a number of isolates of WMV-1 were developed by Purcifull and Hiebert, 1979.

Moroccan watermelon mosaic virus (MWMV)

No data could be found on the biological properties of MWMV. Immunoblot assays are available for MWMV: Yakoubi *et al*., 2007 used DAS-ELISA to characterise a new isolate of MWMV from cucurbits in Tunisia while Miras *et al*., 2019 used ELISA to tests for the MWMV isolate MWMV-SQ10_1.1in twenty four Cucurbit species.

Detection and Identification Methods Based on Viral Nucleic Acid

Cucurbit yellow stunting disorder virus (CYSDV)

The CYSDV genome consists of two molecules of linear, positive sense ssRNA designated RNA1 and RNA2. RNA1 is 9,126 nucleotides long Coutts and Livieratos (2003); RNA2 is 7976 nucleotide (nt) long and contains the hallmark gene array of the family Closteroviridae (Aguilar *et al*., 2003). Rubio *et al*., 2001 performed single-strand conformation polymorphism and nt sequence analyses of the CYSDV coat protein gene to estimate the population structure and genetic variation of CYSDV isolates. Two genetically distinct subpopulations have been identified; the so-called Eastern subpopulation, composed of the Saudi Arabian isolates and the Western subpopulation, containing the rest of the CYSDV isolates. The complete nt sequences of RNAs 1 and 2 of for the Spanish isolate CYSDV-AlLM are known (Augilar *et al*., 2003). Aguilar *et al*., 2003 used in situ hybridisation and RT-PCR using primers to specifically detect negative-sense CYSDV RNA, to investigate the pattern of accumulation of CYSDV RNA in several cucurbit accessions of melon, cucumber, marrow (*Cucurbita maxima*), and squash. In susceptible accessions, CYSDV RNA accumulation peaked at 1-2

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weeks post-inoculation in leaves just below the inoculated leaf, with younger leaves showing very low or undetectable levels of CYSDV. The resistance mechanism is thought to involve a restriction of the virus movement in the vascular system of the plants and/or through prevention of high levels of virus accumulation. Ruiz *et al*., 2001 developed a cost-efficient hybridisation assay to estimate the amount of CYSDV from *B. tabaci* infesting protected cucumber crops. cDNA from the coat protein gene and the hsp70 homologue protein gene from CYSDV were obtained by RT-PCR from viruliferous whiteflies and cloned into plasmids. Digoxigenin (DIG)-labelled cDNA probes reacted with extracts from these whiteflies applied to nylon membranes. The hsp70 probe was then used to evaluate natural *B. tabaci* populations in commercial cucumber crops. To estimate proportions of viruliferous whiteflies in commercial greenhouse-grown crops in Spain, Gil-Salas *et al*., 2007 used RT-PCR to detect and quantify CYSDV in individual adults of *B. tabaci*.

Cucumber Mosaic Virus (CMV)

The genome of CMV consists of linear ssRNA with a total genome size of 8.621 kb. Replication does not depend on a helper virus. RT-PCR methods for detection of CMV were first developed in the early 90's (Rizos, 1992 Blas, 1994). More recently Chen *et al*., 2011 developed a multiplex RT-PCR protocol for simultaneous detection and discrimination of subgroups of CMV, including its satellite RNA using 18S rRNA as an internal control. Aguiar *et al*., 2018 detected and verified the presence of five viruses including CMV and WMV using multiplex RT-PCR. Commercial PCR identification tests are available for this virus through FERA https://www.fera.co.uk/crop-health/virology.

Watermelon mosaic virus (WMV)

The genome of WMV consists of linear, ssRNA with a total genome size of 11.4 kb (Rajbanshi and Ali, 2016). All reported genome sizes of sequences isolates are greater than 10kb (e.g. Desbiez and Lecoq, 2004, Perotto *et al*., 2016). Replication does not depend on a helper virus. Available sequence data has revealed very little genomic diversity amongst WMV isolates. RT-PCR methods for detection of WMV have been available since early 2000s (e.g. Moreno *et al*., 2004). Most recently, Sanchez-Navarro *et al*., 2018 report the development of a unique riboprobe named genus-probe which has the capacity to detect all members of the genus Potyvirus. It carries partial sequences of different plant viruses fused in tandem, and allows for the polyvalent detection of up to 10 different pathogens by using a nonradioactive molecular hybridization procedure.

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Moroccan watermelon mosaic virus (MWMV)

Amino acid and nucleic acid sequence analysis of the coat protein and its cDNA has verified that MWMV is a distinct member of the potyvirus group; McKern *et al*., 1993 examined the relationship of MWMV (then described as WMV-Morocco) using high performance liquid chromatography (HPLC) and found that the coat protein sequence of WMV-Morocco differed substantially from those of WMV2, Miras *et al*., 2019 sequenced the near complete genome of MWMV-SQ10_1.1, a cloned Spanish isolate of MWMV and phylogenetic analysis showed that it shared a common ancestor with other Mediterranean MWMV isolates. Twenty-four Cucurbita spp. accessions were then tested for their susceptibility to MWMV-SQ10_1.1. Progeny analysis suggested that two recessive genes control resistance to MWMV. We hypothesized that this resistance could be associated with alleles of genes encoding the eukaryotic translation initiation factor 4E (eIF4E), particularly after determination of its recessive nature (Miras *et al*., 2019).

Novel technologies

No specific information was discovered on the use of robotics or aerial imagery for the detection of viruses in courgette crops.

Modelling and decision support systems

Decision support systems for courgette growers in the UK are centred around aphid monitoring networks provided by AHDB, Warwick University and Rothamsted Research and aphid predictions based on average winter temperatures. Winter temperature is the dominant factor affecting aphid migration phenologies for *M. persicae* with a 1°C increase in average winter temperature advancing aphid migrations by 4-19 days depending on the species (Zhou *et al*., 1995). This research became central for aphid migration predictions in the UK via Warwick University, AHDB and Rothamsted Research and plays a major part in informing UK courgette growers of the aphid and aphid-borne virus risk. The output from this program is currently available as part of the AHDB Pest Bulletin. For peach-potato aphid (*M. persicae*) monitoring in early March, the Rothamsted Insect Survey produces a forecast of the timing of the migration and the likely relative abundance of peach-potato aphids which can be expected in the early summer. This is based on winter temperatures.

Ruiz *et al*., 2006 investigated the temporal and spatial spread of disease symptoms in plants with mixed infections of CYSDV and CVYV. The authors found that the Gompertz model and logistic model best described the development of CYSDV epidemics, The fitted models were used to calculate the amount of degree Celsius-days at half-maximum infection in greenhouses (°D0·5). 56% of the variation in °D0·5 of CYSDV was related to the numbers of

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whiteflies infesting the cucumber crops, and was independent of the mean temperatures in the greenhouses, while 76% of the variation in °D0·5 of CVYV was related to both the numbers of vectors present and maximum temperature.

Chemical and cultural management options.

Cucurbit virus control is based on three major management practices: (i) planting healthy seeds or seedlings in a clean environment, (ii) interfering with the activity of the viral vectors and (iii) using resistant cultivars. Before the growing season starts, select pathogen-free, high quality seeds. Seed disinfection and seed or seedling quality controls guarantee growers on the sanitary status of their planting material. Aim to plant away from or upwind of other infected fields. Plant early to escape peak aphid season. Handle the plants as little as possible to avoid mechanical transmission. Planting wheat in and around cucurbits can serve as a protection crop, allowing the aphids to wipe the viruses off of their mouthparts before they reach the cucurbit plants. Also, aphids are unable to reproduce on wheat. It may also help to increase water and fertilizer during the growing season. Often courgette plants can outgrow the virus symptoms to go on and produce marketable fruit.

Reflective mulches (Nameth *et al*., 1986, Brown *et al*., 1993; Orozco *et al*., 1994; Summers *et al*., 1995) and mineral oil sprays (Simmon and Zitter, 1980, Zitter 1996) have been used in the past to protect crops from aphids, however aluminium foils are expensive and slow the growth of seedlings, while oil sprays can result in plant injury if done under high temperatures. Courgettes are commonly grown through polythene mulch as there are very few herbicides authorised for use in the crop. This method is very effective in controlling most annual weeds within the rows. However, weeds can grow through the holes, and controlling weeds in the inter-row areas between the mulch will become difficult after the loss of diquat, which was the main method of post-planting weed control in cucurbits. Growers are investigating alternative methods of weed control between rows such as living mulches. Hand weeding is an option, but is expensive.

Another type of mulch which is occasionally used on organic farms is compost or woodchip and this is being evaluated in an EIP project on a Welsh organic vegetable farm (2018-19) for Horticulture Wales. It is also being evaluated by a current Innovative Farmers group. Cucurbit growers are evaluating straw as a mulch between rows, in addition to dwarf rye to outcompete weeds in the inter-row areas. This could be investigated for other wide row crops (Cook *et al*., 2019).

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Breeding for aphid resistance and host resistance and/or tolerance

Resistance can be obtained by grafting for soil-borne viruses, by cross-protection, or generally by conventional breeding or genetic engineering. Within the Cucurbitaceae family, the complete genome of cucumber and the draft genome of courgette are already available (Huang *et al*., 2009; Garcia- Mas *et al*., 2012; Guo *et al*., 2013). Resistance to CYSDV in melon is reported to be controlled by a single dominant gene in the breeding line TGR 1551 (López-Sesé and Gómez-Guillamón, 2000). McCreight and Wintermental (2008) evaluated genetically diverse melon cultigens for resistance to CYSDV, with Melon Plant Introduction (PI) 313970 expressing high-level resistance to CYSDV. Partial resistance to CYSDV has also been described in cucumber by Eid *et al*., 2006.

Among cucurbits, resistance to CMV is thought to be quantitatively inherited and mostly recessive (Shi *et al*., 2018). Zhou, 2005 reported that resistance to CMV in squash is due to a major gene as well as a recessive gene, however Celai *et al*., 2014 reported that resistance is governed by one gene and at least two QTLs. Shi *et al*., 2018 studied resistance to CMV using a RIL of cucumber `02245', to identify QTLs and candidate genes linked to CMV resistance. Nine genes were found to be related to disease resistance within the CMV mapping region. The proximity of the genetic distances between the resistance genes indicated that virus resistance genes in cucumber are located within the same gene cluster. Vat is a unique gene conferring resistance to both *A. gossypii* and the viruses it carries. However the effect of Vat on virus epidemics in the field is expected to vary according to the composition of aphid populations in the environment. Schoeny *et al*., 2017 monitored epidemics of CABYV, CMV, WMV and ZYMV in a resistant and susceptible line of melon having a common genetic background. Vat a favourable impact on CMV, yet of variable intensity probably related to the importance of *A. gossypii* in the total aphid population.

Anagnostou *et al*., 2000 demonstrated dominant monogenic inheritance of resistance to WMV in a segregating population of melon breeding line was observed. Line 414723-4S3, which was initially selected as a source of ZYMV and WMV resistance, is also a source of dominant monogenic resistances to PRSV and PM race 1. The authors also found genetic linkage between resistance to WMV and ZYMV.

Inbred lines derived from the Chinese cucumber cultivar, Taichung Mau Gua (TMG), have been identified as resistant to several potyviruses including ZYMV, ZPRSV-W, WMV and MWMV (Gilbert-Albertini *et al*., 1995; Kabelka and Grumet, 1997; Provvidenti, 1985; Wai and Grumet, 1991, 1995). Kabelka *et al*., 1996 showed that TMG-1 resistance to MWMV is conferred by a single recessive gene. Sources of resistance to MWMV have been identified

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in cucurbit species *Cucumis metuliferus, Citrullus ecirrhosus, Coccinia sessifolia and Luffa aegyptica* (Provvidenti & Hampton, 1992) but not for *C. sativus*.

Genetic modification and CRISPR technology

Agrobacterium mediated transformation has been accomplished in all of the three Cucurbitaceae genera with regeneration performed through shoot organogenesis, however transformation efficiency has been very genotype dependant (Klocke *et al*., 2010; Manamohan *et al*., 2011). Improvements in the methodology have been reported for cucumber (Wang *et al*., 2015) and other Cucurbita spp. (Nanasato *et al*., 2013). Attempts to develop *in planta* methods (either via pollen tube or microinjection of the shoot apical meristem) have been made in cucumber (Baskaran *et al*., 2016). A simple dominant control of regeneration ability from leaf explants has been found in cucumber (Nadolska-Orczyk and Malepszy, 1989), while distinct expression profiles of WUSCHEL related homeobox (WOX) genes have been associated with different regeneration abilities in watermelon (Zhang *et al*., 2015).

Virus resistance of cucumber plants has been investigated a using Cas9/subgenomic RNA (sgRNA) technology to disrupt the function of the recessive eIF4E gene at two sites; the first by complete gene knock-down and the second, where translation of two-thirds of the protein product was still possible (Chandrasekaran *et al*., 2016). Small deletions and SNPs were observed in the eIF4E gene targeted sites of transformed T1 generation cucumber plants, but not in putative off-target sites. Non-transgenic homozygous mutant plants showed either immunity or resistance to *Cucumber vein yellowing virus* (CVYV), *Zucchini yellow mosaic virus* (ZYMV), and *Papaya ring spot mosaic virus- W* (PRSV-W), although resistance breaking was observed in some cases. The same plants had no resistance to CMV and *Cucumber green mottle mosaic virus* (CGMMV). This was the first report of virus resistance developed in cucumber non-transgenically, which did not visibly affect plant development and without long-term backcrossing.

Criniviruses are notoriously difficult to purify and traditional methods of antibody production, requiring purified virus particles, are extremely challenging. Steel *et al*., 2010 describe the development of a strategy for *in planta* viral antigen preparation to bypass particle purification and allow antibody production. *A. tumefaciens*-mediated transient expression, coupled with GFP purification was employed to generate CYSDV coat protein in whole plant leaves of *N. benthamiana*. Expression levels of the recombinant protein were increased by co-infiltration with the viral gene-silencing suppressor P19 from TBSV. The recombinant protein purified from plant leaves was used to immunise rats for the preparation of polyclonal antisera. Viral satellite RNA associated with CMV is known to modulate CMV symptomology. Naturally

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occurring variants of these satellites, however, attenuate CMV symptoms. Stommel *et al*., 1996 evaluated satellite transgenic tomato plants expressing the S-CARNA 5 or 1-CARNA 5 ameliorating forms of the satellite demonstrated that CMV can be effectively controlled under field conditions in satellite transgenic plants, with expression of CARNA 5 in inoculated transgenic plants greatly reducing CMV foliar symptoms. To characterise the genetic structure of CMV populations, Garcı´a-Arenal- *et al*., 2000 compared ca. 300 isolates of CMV, representing 17 sub-populations. Genetic analyses of CMV isolates by ribonuclease protection assay of cRNA probes representing RNA1, RNA2 and the two open reading frames in RNA3 showed that all isolates belonged to one of three genetic types: Sub-group II and two types of Sub-group I. About 30% of CMV isolates were supporting a satRNA. The CMV-satRNA population has a genetic structure and dynamics different from those of its helper virus. This indicates that CMV-satRNA has spread epidemically on the extant virus population from an original reservoir in eastern Spain.

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Virus management in lettuce

Wholehead lettuce is grown on a wide range of soils and is usually transplanted, with 4,391 ha planted in the UK in 2017 (Defra, 2018). Plant spacing varies by type e.g. little gem will be planted closer together than iceberg. Baby leaf includes a range of salad crops with different species (such as lettuce, chard, spinach and wild rocket), and 1,837 hectares were grown in 2017 (Defra, 2018). All crops are harvested before eight true leaves, which defines the herbicide authorisations and products for use on these crops.

This review builds on that conducted in AHDB Horticulture Project FV427 'Outdoor lettuce: Screening crops for presence of virus' (McPherson *et al*., 2014). This review identified 61 viruses with the potential to infect lettuce. Thirteen of these 61 viruses have been identified on lettuce in the UK or are likely to be present due to their strong association with other viruses.

Industry feedback thus far has been that viruses are indeed a problem in lettuce crops, but these are very often misdiagnosed as magnesium deficiency or Odema (Gaffney, M. Pers. Comm.). CuMV and LMV are the primary issues for lettuce growers in the UK and if sugar beet also has no seed neonicotinoid treatment, growers are concerned about transmission of virus from this crop. Loss of pymetrozine further reduces aphid control potential (Norman, D. Pers. Comm.).

The present review will focus on four of these viruses currently present in the UK. For further information on *Beet pseudo yellows virus, Beet western yellows virus* and *Beet yellow stunt virus* see the section on sugar beet. For further information on *Cucumber mosaic virus* see the section on cucurbits. For further information on *Tomato spotted wilt virus* see the section on tomato and for further information on *Turnip mosaic virus*, see the section on oilseed rape and vegetable Brassicas.

Current viruses present in the UK

In addition to the review conducted by McPherson *et al*., 2014 in FV427, the project team also conducted a baseline study of geographically diverse lettuce crops from July to September 2014 which identified nine viruses, four of which had not been reported previously. The key findings were that no apparent correlation could be found between virus incidence and samples declared as asymptomatic by growers. Virus incidence was not associated with variety, but instead likely to geographical location. The report could not give any clear strategies to mitigate against viral spread and development aside from methods currently employed and indicated that further research should be done to understand carry over, transmission and identification of virus reservoirs.

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Table 29. Summary of viruses reported on lettuce in the United Kingdom, together with, where available, information on their mode of transmission and their vector. Viruses highlighted in bold italics are discussed below.

Virus	Acronym	Genus	Mode of transmission
Arabis mosaic virus	ArMV	Nepovirus	Nematode (e.g. *Xiphinema diversicaudatum*), seed transmission
Beet pseudo yellows virus	BPYV	Closterovirus	Whitefly (*T. vaporariorum*)
Beet western yellows virus	BWYV	Luteovirus	Aphids (e.g. *M. persicae*)
Beet yellow stunt virus	BYSV	Closterovirus	Aphids (e.g. *H. lactucae*)
Cucumber mosaic virus	CMV	Cucumovirus	Aphids (e.g. *M. persicae*)
Dandelion yellow mosaic virus	DYMV	Sequivirus	Aphids (e.g. *M. persicae*)
Lettuce big-vein associated virus	LBVaV	*Varicosavirus*	*Olpidium brassicae*
Lettuce mosaic virus	LMV	*Potyvirus*	*Aphid (A. gossypii, Macrosiphum euphorbiae, M. gei, M. persicae mechanical inoculation, seed*
Lettuce necrotic yellows virus	LNYV	*Cytorhabdovirus*	*Aphid (Hyperomyzus lactucae)*
Lettuce ring necrosis virus	LRNV	Ophiovirus	*Olpidium brassicae*
Mirafiori lettuce big-vein virus	MiLBVV	Ophiovirus	*Olpidium brassicae*
Tomato spotted wilt virus	TSWV	Tospovirus	Thrips (e.g. *F. occidentalis*)
Turnip mosaic virus	TuMV	Potyvirus	Aphids (e.g. *M. persicae*)

Lettuce big vein disease: Lettuce big-vein associated virus (LBVaV) and Mirafiori lettuce big-vein virus (MiLBVV)

Big-vein disease of lettuce was first reported in California by Jagger and Chandler 1934. It occurs in cool to temperate and occasionally subtropical regions and is a serious problem for lettuce production during cooler periods of the year (Barcala Tabarrozzi *et al*., 2010; Huijberts

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et al., 1990; Roggero *et al*., 2000; Vetten *et al*., 1987). Infected lettuce plants develop vein bandings and ruffled, distorted leaves, reducing market value (Sasaya, 2013). A rod-shaped virus called *Lettuce big-vein associated virus* (LBVaV; genus Varicosavirus), was initially thought to be the causal agent of big-vein disease (Kuwata *et al*., 1983; Walsh & Verbeek, 2011), until a filamentous virus *Mirafiori lettuce big-vein virus* (MiLBVV, genus Ophiovirus), was isolated from big-vein-affected lettuce plants (Roggero *et al*., 2000). A number of studies have since confirmed that MiLBVV, but not LBVaV, induces big-vein symptoms in lettuce (Lot *et al*., 2002; Roggero *et al*., 2000; Sasaya *et al*., 2008). However, many studies have demonstrated that plants exhibiting big vein symptoms are often co-infected with both viruses, suggesting that LBVaV has a role in the development of the disease (Navarro *et al*., 2004, 2005, Roggero *et al*., 2003).

Lettuce Mosaic Virus (LMV)

LMV, a member of the Potyvirus family of viruses (Moreno *et al*., 2007) is the causal agent of lettuce mosaic, the most devastating viral disease of lettuce worldwide (Dinant & Lot, 1992). The virus was first reported in lettuce from Florida, U.S.A by Jagger (1921). Symptoms include necrotic or chlorotic local lesions and streaking, then mosaic, vein yellowing, leaf malformation on Lactuca spp., other symptoms may include leaf chlorosis and chlorotic mottling. Symptoms caused by LMV can vary considerably depending on the genotype of the host plant, infective strain or stage of infection and environmental conditions (Dinant and Lot, 1992). Three pathotypes of the virus are recognised (Pink, Kostova *et al*. 1992); (Moura *et al*., 2018) report on the high biological variability of LMV isolates, which can be classified into two large groups known as LMV-Common, which infects susceptible lettuce cultivars, and LMV-Most, which is more aggressive, capable of being transmitted through seeds and can break down the recessive inherited resistant alleles *mol1* and *mol2* (Nicaise *et al*., 2003). In Brazil, LMV-resistant lettuce cultivars have been found to be tolerant to LMV-Common isolates, including LMV pathotype II, according to the classification of Dinant & Lot (1992). Pavan *et al*., 2008 report on the lack of lettuce cultivars that are resistant or tolerant to LMV-Most and highlight the need to search for possible new sources of LMV-Most resistance.

Lettuce necrotic yellows virus (LNYV)

LNYV is a cytorhabdovirus and a member of the family Rhabdoviridae family of viruses (Verbeek *et al*., 2017). It causes a severe disease of lettuce in Australia and New Zealand, with the first reports in 1963 and 1973 (Stubbs *et al*., 1963). There also have been isolated reports of a similar virus infecting lettuce in Spain, Italy and Great Britain (Moreno and Fereres, 2012). Symptoms on lettuce leaves first resemble a dull green which turn chlorotic. Outer leaves can be flaccid, bronzed and sometimes necrotic, plants can be stunted and late

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infected plants have heads with internal necrosis. Both severe (e.g. SE3) and mild strains of this virus have been reported by Stubbs and Grogan (1963).

New emerging viruses

Plant Virus Online lists 53 viruses which can infect lettuce (Brunt et al., 1996). Table 30 lists a selection of these viruses which are of particular interest due to their reported virulence on lettuce and or widespread presence in Europe.

Table 30. Potential emerging viruses. These viruses were identified in FV427 as occurring naturally on lettuce outside the UK and of particular interest due to their virulence on lettuce and or widespread presence in Europe.

Virus	**Acronym**	**Genus**
Alfalfa mosaic virus	AMV	Alfamovirus
Broad bean wilt virus	BBWV	Fabavirus
Endive necrotic mosaic virus	ENMV	Potyvirus
Lettuce necrotic stunt virus	LNSV	Tombusvirus
Tobacco rattle virus	TRV	Tobravirus

Vectors of key viruses

Lettuce big vein disease: Lettuce big-vein associated virus (LBVaV) and *Mirafiori lettuce big-vein virus (MiLBVV)*

Both LBVaV and MiLBVV are transmitted by *Olpidium brassicae*, a fungus of the order Chytridiales. These virus are not transmitted by insect species.

Lettuce Mosaic Virus (LMV)

LMV is primarily seed borne; secondary infection occurs through aphids including *A. gossypii, M. euphorbiae, M. gei and M. persicae* in a non-persistent manner using the helper component strategy (Ng and Perry, 2004, Sharma *et al*., 2016). The virus can also be transmitted by mechanical inoculation and by pollen to the seed (Ryder, 1973; Dinant & Lot, 1992; Moreno *et al*., 2007). Vectors of LMV can be distinguished and grouped by their mode of action; those that land but do not reproduce in the crop; transient vectors (e.g. *M. persicae*), transient nonvectors (e.g. *H. pruni*) colonising vectors (e.g. *M. euphorbiae*) and colonising non-vector species (e.g. *N. ribis-nigri*) (Kennedy *et al*., 1962; Blackman & Eastop, 1985; Nebreda *et al*., 2004).

Lettuce necrotic yellows virus (LNYV)

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LNYV is transmitted persistently in a propagative manner, primarily by the blackcurrant-sowthistle aphid (*H. lactucae)* in a persistent manner. The currant-lettuce aphid *N. ribis-nigri* is the most significant pest infesting lettuce in northern Europe (Collier *et al*., 1999; Reinink and Dieleman 1993). The virus is retained when the vector moults; it multiplies in the vector and is transmitted congenitally to the progeny of the vector. It can also be transmitted by mechanical inoculation. This virus is not transmitted by seed. Transmission by *H. carduellimus* (Theob) appears less common due to its limited geographical distribution.

Table 31. Vectors of key viruses of lettuce

Virus	**Vector**
LBVaV and MiLBVV	*Olpidium brassicae*
LMV	*Aphis gossypii, M. euphorbiae, M. gei, M. persicae*
LNYV	*H. lactucae, H. carduellimus, B. tabaci*

Alternate hosts of key viruses

Lettuce big vein disease: Lettuce big-vein associated virus (LBVaV) and *Mirafiori lettuce big-vein virus (MiLBVV)*

The weed species *Chenopodium spp., Nicotiana spp., Sonchus spp., Tetragonia tetragonioides are known hosts of both LBVaV and MiLBVV (*Brunt et al., 1996*).*

Lettuce Mosaic Virus (LMV)

Several herbaceous ornamental plant and weed species such as *Sonchus* spp., *S. vulgaris* L. or *L. serriola* L. can be potential reservoirs and inoculum sources of LMV (Tomlinson, 1970; Moreno *et al*., 2004). Plant Virus Online also lists *Amaranthus caudatus Beta vulgaris Capsella bursa-pastoris Carthamus tinctorius Chenopodium spp. Cicer arietinum Cichorium endive Gomphrena globose Lathyrus odoratus Nicotiana spp. Pisum sativum Senecio vulgaris Sonchus Spinacia oleracea Stellaria media Tetragonia tetragonioides* and *Zinnia elegans* as susceptible to LMV (Brunt et al., 1996).

Lettuce necrotic yellows virus (LNYV)

Sowthistle (*S. oleraceus*) is recognised as the most important reservoir host. In addition to lettuce, field infections have been reported in a number of species including garlic (*Allium sativum*) and chickpea (*Cicer arietinum*). Plant Virus Online also lists *Datura stramonium Embergeria megalocarpa Gomphrena globose, Lycopersicon esculentum Nicotiana spp., Petunia × hybrida Reichardia tingitana Sonchus spp. and Spinacia oleracea* as susceptible

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to LNYV (Brunt et al.,1996). For a full list of weeds which are susceptible to LNYV, see Chu and Francki (1982).

Table 32. Alternate hosts of key viruses

Virus	Alternate host species
LBVaV and MiLBVV	*Chenopodium spp., Nicotiana spp., Sonchus spp., Tetragonia tetragonioides*
LMV	*Amaranthus caudatus, Beta vulgaris, Capsella bursa-pastoris, Carthamus tinctorius, Chenopodium spp., Cicer arietinum, Cichorium endive, Gomphrena globose, Lathyrus odoratus, Nicotiana spp., Pisum sativum, Senecio vulgaris, Sonchus, Spinacia oleracea, Stellaria media, Tetragonia tetragonioides, Zinnia elegans*
LNYV	*Datura stramonium, Embergeria megalocarpa, Gomphrena globose Lycopersicon esculentum, Nicotiana spp., Petunia × hybrid, Reichardia tingitana, Sonchus hydrophilus, Sonchus oleraceus, Spinacia oleracea*

Detection and Identification Methods Based on Biological Properties

AHDB Horticulture Project FV427 identified that high throughput ELISA reagents are available for LMV and MiLBVV. No reagents for ELISA assays were found for LBVaV or LNYV. For a full list of reagents available for individual viruses see McPherson *et al.*, (2014).

Lettuce big vein disease: Lettuce big-vein associated virus (LBVaV) and *Mirafiori lettuce big-vein virus (MiLBVV)*

The virions of LBVaV consist of a capsid which is not enveloped. The capsid is rod-shaped and straight with a clear modal length with a length of 320-360 nm and a width of 18 nm. Virus preparation contains few virions. Aldehyde fixation is essential for electron microscopy (Kuwata *et al*., 1983). The density of virion of LBVaV is 1.27 g cm^{-3} in Cs2SO4. Immuno-based assays are available for the detection of both LBVaV and MiLV: Colariccio *et al*., 2003 tested lettuce samples collected in different areas of Sao Paulo, Brazile by DAS-ELISA with antisera specific for MiLV and LBVV (Roggero *et al*. 2003) and also examined tissues by electron microscopy (EM) using negative staining for the presence of viruses. Most samples with big-vein symptoms tested positive by ELISA for both viruses and a few only for one of the two. Using EM, only LBVV virus particles were observed; virions were observed in only a few samples confirming the difficulty in EM visualization of both LBVV and MiLV particles in the same sample.

Lettuce Mosaic Virus (LMV)

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Leaf sap containing LMV contains few virions. Virions present are filamentous, non-enveloped and usually flexuous with a modal length of 680-900 nm long and 11-15 nm wide (Moreno *et al*., 2007). The density of LMV is 1.33 g cm^{-3} in sucrose. Experimental infection with viruliferous aphids has been reported for both lettuce and sowthistle, while infection by mechanical inoculation has been reported for species such as *N. glutinosa*, N. clevelandii, *P. hybrida* and *D. stramonium* (Chu and Francki, 1982). ELISA based assays are available for LMV diagnosis; most recently Sharma *et al*., 2016 used a PTA-ELISA method for highly accurate specific detection of LMV in lettuce accessions. In the UK FERA offer a commercial service to growers to test both seeds stocks and leaf material for presence of LMV https://www.fera.co.uk/lettuce-mosaic-virus-by-elisa.html.

Lettuce necrotic yellows virus (LNYV)

Leaf sap containing LNYV contains variable numbers of virions. Virions present are rhabdo- or bullet-shaped and enveloped, with a length of 227 nm x 66nm wide in negative stain or 360 nm x 52 nm wide in tissue sections. Infectivity of LNYV is lost when de-proteinised with phenol or detergent. Aldehyde fixation is necessary for electron microscopy. N. glutinosa is used as the experimental host for LNYV biological indexing, maintenance, propagation and purification. The density of LNYV is 1.19-1.2 g cm^{-3} in sucrose or ca. 1.135 g cm^{-3} in Nycoden. ELISA based methods for detection of LNYV have been available since the 1980s (Chu and Francki, 1982). Dietzgen and Francki (1990) describe a procedure for the detection of LNYV nucleocapsid protein or envelope glycoprotein by immuno-blotting with their respective monoclonal antibodies from fresh tissue of systemically infected *Nicotiana glutinosa* leaves showing prominent symptoms. The authors found that reducing agents such as 2-mercaptoethanol (2-ME) or dithiothreitol (DTT) interfered with the detection process.

Detection and Identification Methods Based on Viral Nucleic Acid

Lettuce big vein disease: Lettuce big-vein associated virus (LBVaV) and *Mirafiori lettuce big-vein virus (MiLBVV)*

Both LBVaV and MiLBVV have segmented ssRNA genomes. The viral RNA of LBVaV is predominantly negative-sense whereas MiLBVV particles contain approximately equimolar amounts of RNA molecules of both polarities (Alemzadeh and Izadpanah 2012). The LBVaV genome contains two RNA segments. Amasi, 2017 developed a RT-LAMP assay for detection of MiLBVV compared the findings to DAS-ELISA and RT-PCR, considering factors such as safety, simplicity, cost, user-friendliness and safety. RT-LAMP and IC-RT-LAMP had higher sensitivity (100-fold) than DAS-ELISA and RT-PCR but similar specificity, with the advantage of a shorter assay time and no need for RNA extraction (in IC-RT-LAMP). As RT-

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LAMP requires only very basic instruments and the results can be obtained by visual inspection, this technique provides a simple and reliable tool for laboratory based research.

Lettuce Mosaic Virus (LMV)

The genome of LMV is positive-sense ssRNA with a total genome size of 10.08kb (German-Retana *et al*., 2008). It is expressed as a polyprotein that cleaves to functional proteins (López-Moya and García, 1999; Hull, 2002). RT-PCR as a detection method for LMV has been available since the late 1990's with the first report made available by Revers *et al*., 1997 who used RT-PCR to amplify and compare sequences of 10 different isolates of LMV, selected to cover the biological and geographical diversity of LMV in Europe. Peypelut *et al*., 2004 used RT-PCR for the specific detection of LMV-Most isolates. The assays were performed against a collection of 21 isolates from different geographical origins and representing the molecular variability of LMV. RT-PCR of the central region of the genome was preferred because its results are expected to be less affected by natural recombination between LMV isolates, and it allows sensitive detection of LMV-Most in situations of single as well as mixed contamination. Moreno *et al*., (2007) used two PCR-based methods with a previous immuno-capture phase to detect LMV in single aphids of *M. persicae* (vector species) and *N. ribis-nigri* (non-vector species). Although the percentage of viruliferous aphids for *N. ribis-nigri* was higher than for *M. persicae* after the same acquisition access period, *N. ribis-nigri* was unable to transmit the virus while *M. persicae* proved to be an efficient vector. Attempts to link the key biological properties of LMV isolates by sequence clustering have been carried out (Krause-Sakate *et al*., 2002; Revers *et al*., 1997). These studies established a link between biological properties and sequence clustering, and provided the bases for strain-specific detection of LMV, once the complete nucleotide sequence of an LMV-Most isolate had been established (Krause-Sakate *et al*., 2002; Peypelut *et al*., 2004). The molecular variability of LMV isolates was also revealed, using monoclonal antibodies directed against the coat protein, but these studies could not reveal any difference between LMV-Common and LMV-Most, probably owing to their identical amino-acid sequence in the immunogenic N-terminus of the coat protein (Candresse *et al*., 2007).

Lettuce necrotic yellows virus (LNYV)

The genome of LNYV consists of ssRNA with a total genome size 12.54 kb. Three major structural proteins of *lettuce necrotic yellow virus* (LNYV) were identified by discontinuous polyacrylamide gel electrophoresis (PAGE) (Dietzgen and Francki, 1988). Verbeek *et al*., (2017) constructed protein localization and interaction maps of the associations of viral structural and non-structural proteins and the mechanisms by which they may function in LNYV infection Subcellular localization was determined by transiently expressing the viral

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proteins of LNYV fused to green or red fluorescent protein in leaf epidermal cells of *N. benthamiana*. Protein interactions were tested in planta by using bimolecular fluorescence complementation. This is the first report of protein localization and interactions for a Cytorhabdovirus.

Novel technologies

Van Der Weide (2008) produced a detailed review of how sensing technologies differ. Vision Robotics' technology reportedly integrates algorithms with sensor technology to bring automation to lettuce farming and vineyards. Specifically, computer vision allows robots to generate 3D maps and models of areas of interest and then to complete various tasks within those parameters (Emerj, 2017).

Modelling and decision support systems

Spatial analysis by distance indices (SADIE), has been developed by Perry (1995, 1998) to quantify the spatial pattern in a sampled population by measuring the distance individuals must move to give an extreme arrangement in which the individuals in the samples are spaced as uniformly or regularly as possible (Moreno *et al*., 2007). The SADIE methodology has been used together with mathematical models for analysis of disease progress curves to understand the driving forces involved in the spread of plant viruses in several crops (Alonso Prados *et al*., 2003; Jones, 2005, Lathman & Jones, 2001).

Decision support systems for lettuce growers in the UK are centred around aphid monitoring networks provided by AHDB, Warwick University and Rothamsted Research and aphid predictions based on average winter temperatures.

The currant-lettuce aphid (*N. ribis-nigri*) and lettuce-root aphid (*P. bursarius*) are captured in the network of suction traps operated by the Rothamsted Insect Survey. They can also be captured in yellow water traps and commercial monitoring services using water traps are available. A forecast system developed at the University of Warwick is based on accumulated day-degrees (D°) from 1st February (base 4.4°C). Information from the Rothamsted Suction trap captures at Wellesbourne and Kirton is used to estimate the mean number of D° until the first aphid of the year is caught in a suction trap (the start of the migration to lettuce). This is after approximately 507°D for the currant-lettuce aphids and 672°D for the lettuce-root aphid. The output from this program is currently available as part of the AHDB Pest Bulletin. For peach-potato aphid (*M. persicae*) monitoring in early March, the Rothamsted Insect Survey produces a forecast of the timing of the migration and the likely relative abundance of peach-potato aphids which can be expected in the early summer. This is based on winter temperatures.

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Chemical and cultural management options.

The principles of virus management as detailed by Fox *et al*., 2017 in AHDB Horticulture project FV453 are applicable across multiple crops and cropping scenarios. The section on potato virus management details the use of straw mulching, crop borders and mineral oil spraying for effective management of viruses and their associated vectors on seed and ware potatoes and the Section on OSR discuss the suitability of biopesticides and physical barriers such as fleeces, nets, or insect traps for vector management.

Management of viruses and their vectors in lettuce crops starts from pre-planting, through to propagation in the nursery, transplanting, vegetative growth all the way through to harvest.

Pre planting

Rotations out of lettuce will help reduce big vein disease incidence. Weed control is essential in and around production areas. Collectively, the viruses infecting lettuce have a very wide range of potential hosts so a thorough weed management program is recommended, rather than selecting individual species which may be common hosts. Reimans *et al*., 2007 used a covered rotary harrow during the day prior to drilling lettuce, this was effective in reducing weed levels by 17% in two out of three years in a stale seedbed and by 60% during plant bed preparation, with the differences in control between years attributed to different dormancy states. Mechanical hoeing is widely used as there are very few authorisations for the crop. There are many studies which evaluated the damage caused by flame weeding in a range of crops including; lettuce and white cabbage (Balsari *et al*., 1994; Netland *et al*., 1994). Current research investigating the benefits of flame weeding in vegetable systems is being investigated in a European H2020 funded project IWMPraise (2016). Purchase seed and seedlings from reliable suppliers and ensure that it has been tested for LMV freedom. Broadbent *et al*., 1951 showed that the use of virus-tested seeds is the most effective way of reducing the incidence of LMV. Consider LBVD tolerant varieties when growing during cool weather in areas with a history of the disease. If aphid-transmitted viruses are an ongoing issue (CMV, LMV and TuMV) consider the use of permanent wind rows and strategic planting to maximise the distance between lettuce crops.

Nursery

Ensure that the nursery area is free of weeds. Where possible locate the nursery in a remote area, away from production areas.

Transplant through to vegetative growth and harvest

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Maintain weed control in and around production areas, especially of virus host plants. Remove any virus affected plants from the crop area to minimise virus spread. Avoid planting new crops near old crops, particularly where virus and other diseases have been prevalent. It is important to reduce virus spread to subsequent crops. Avoid planting near affected crops and plant upwind. Have non-host crops as alternate plantings.

Current aphid control measures, are based around a number of active substances including pirimcarb, acetamiprid*, Beauvaria bassiana* and pymetrozine under protection for *M. persicae*.

Lettuce should be monitored for insect vectors as soon as the plants emerge, or go out into the field if using transplants. Growers should pay particularly close attention to lettuce planted downwind or adjacent to these fields. Once whitefly adults appear in a field in sufficient numbers, treatments should begin. Whiteflies are best controlled by preventing colonization; do not allow adults to build and lay eggs. Monitor for whiteflies early in the morning when the adults are sedentary. Once temperatures begin to increase, the adults will begin to stir and move, and they will become difficult to count. Mid-morning, monitor movement by looking for dispersing swarms (Anonymous, 1987).

Green peach aphid (also known as peach-potato aphid (*Myzus persicae*)) prefers the underside of the older leaves. After thinning and before heading, colonies of ten or fewer aphids, can be tolerated. Once lettuce nears head formation, green peach aphids cannot be tolerated. The key to controlling green peach aphid is to prevent the formation of large colonies (Palumbo and Kerns, 1995). Adequate control is often difficult to achieve with foliar sprays, and follow-up scouting should be performed to determine if another insecticide application is necessary. Green peach aphids are often most numerous in fields containing weedy mustards and members of the goosefoot family. Control of these weeds may help prevent buildup of green peach aphid. Once virus is present in a crop, it is too late to eradicate the virus and attempts to control aphid vectors at this point can further spread virus in the crop for diseases such as CMV and LMV, due to the method of virus transmission (Kerns, et al., 1995).

Hearting through to harvest

If the virus is observed in the last stages of crop growth, there will be a minimal effect on yield. Destroy whatever is left in the field after harvest promptly. Do not plant new crops close to old crops. Plan to have fallow land or non-susceptible crops as a means of separating plantings. Remove any virus-affected plants from the crop area to minimise virus spread to other plants, particularly younger plantings that are nearby.

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Breeding for aphid resistance and host resistance and/or tolerance

Cultivated lettuce is a self-fertilizing diploid species from the family of Compositae (Asteraceae). The first draft of the lettuce genome was made available by Galla *et al*., 2014, paving the way to speedier mapping, cloning, and functionally validating genes for disease resistance and for more efficient development of molecular markers used in MAS (Simko, 2013). Walley *et al*., 2017 describe the publicly available UK Vegetable Genetic Improvement Network lettuce diversity set; a valuable breeding resource that captures wide genetic variation in *Lactuca* species and has a diverse range of morphological variation mediated by an associated panel of NGS-derived single nucleotide polymorphism (SNP) markers. This population is accompanied by a panel of breeder-friendly lettuce-specific KAS markers that have been anchored in the *L. sativa* genome assembly and are amenable to cost effective high-throughput genotyping or as smaller subsets for MAS. The value of the diversity set for lettuce breeding research has been demonstrated by phenotyping for resistance to *N. ribisnigri* biotype Nr:0, and several LKAM/ESTs associated with observed resistance have been nominated.

Partial resistance to MLBVV (Jagger and Chandler, 1934) was identified in butterhead lettuce while *Lactuca virosa* accession IVT 280 appeared to be immune (Hayes *et al*., 2006). However, RT-PCR analysis demonstrated the presence of the virus in asymptomatic plants of these accessions (Hayes *et al*. 2008). A population developed from crossing susceptible butterhead cv. Parade with cv. Pavane identified one chromosomal region on LG 3 and two regions on LG 4 that contribute to resistance to MLBVV. The three QTLs together explained 56% of the observed phenotypic variation (Hayes *et al*. 201a; Michelmore, 2010). Two different QTLs were mapped on LG 5 and LG 6 in a population developed from a cross between a resistant and susceptible iceberg type lettuce cvs. (Michelmore, 2010), indicating that these cvs. represent different sources of resistance to MLBVV, thus a higher level of resistance could be achieved by combining QTLs from multiple sources.

Four genes have been reported to confer resistance to LMV; one recessive gene (*mo-1*) and three dominant genes (*Mo-2, Mo-3,* and *Mo-4*) (Candresse *et al*., 2006). To the authors knowledge, the dominant genes are currently not used in breeding programs because of their limited durability (Mo-2) (Pink *et al*., 1992); or the difficulty of introgressing genes from wild species (Mo-3 and Mo-4) into cultivated lettuce (Le Gall *et al*., 1999; Maisonneuve *et al*., 1999; Candresse *et al*., 2006). The recessive mo-1 gene is usually associated with tolerance or resistance to LMV (Dinant and Lot, 1992), depending on the virus isolate and genetic background of the plants (Pink *et al*., 1992; Revers *et al*. 1997). *mo1* encodes eIF4E. The eIF4E–CI interaction reported by (Tavert-Roudet *et al*., 2012) is thought to contribute to the cell-to-cell movement process, thus potentially explaining the resistance phenotypes

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sometimes associated with eIF4E-mediated resistance. Data from (Tavert-Roudet *et al*., 2012) and previous studies suggest that the C-terminal domain of the LMV CI protein could be involved in a large interaction network of viral and cellular proteins; this network and the underlying function(s) that are associated with the viral and host protein interactions involved is still to be fully understood. The mo-11 allele is frequently used in European breeding programs (Pink *et al*., 1992) while the mo-12 allele was used to develop LMV-resistant cultivars in the USA (e.g., Ryder 1973). Sequencing of mo-1 confirmed that mo-11 and mo-12 are alleles of the same gene that encodes eIF4E (Nicaise *et al*., 2003).

Genetic modification and CRISPR technology

Lettuce has been successfully used in many transformation experiments for a variety of purposes, using either Agrobacterium inoculation of various explants (mainly cotyledons and true leaves) or direct gene transfer (electroporation/PEG treatment of protoplasts or particle bombardment of tissue explants) (Davey *et al*., 2007; Klocke *et al*., 2010; Song *et al*., 2014; Matvieieva, 2015). Shoot regeneration from hairy roots has also been accomplished. Regeneration normally proceeds by organogenesis, but SE has also been reported. Recently, the “surface response” method has been employed in lettuce to optimize plant regeneration (Gómez-Montes *et al*., 2015). An alternative innovative approach for delivering editing reagents in plant cells has been recently reported in a number of species, including lettuce (Woo *et al*., 2015). The homolog of the Arabidopsis BRASSINOSTEROID INSENSITIVE 2 (BIN2) gene, encoding a negative regulator in the brassinosteroid (BR) signalling pathway was knocked out after transfecting PEG-treated protoplasts with a mixture of Cas9 and a sgRNA targeting the third exon of the gene. Overall mutation frequency in protoplast-derived calli was 46%. No off-target mutations were detected and plants regenerated via organogenesis from mutant calli transmitted the mutations to the progeny. Sasaya, 2013 conducted microprojectile bombardment experiments to identify a movement protein gene of MiLBVV. A plasmid containing an infectious clone of a *tomato mosaic virus* (ToMV) derivative expressing the GFP was co-bombarded with plasmids containing one of three genes from MiLBVV RNAs 1, 2 and 4 onto *N. benthamiana*. Intercellular movement of the movement-defective ToMV was restored by co-expression of the 55 kDa protein gene, but not with the two other genes. The 55 kDa protein encoded in the MiLBVV RNA2 functions as a movement protein of the virus.

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Virus management in alliums

Alliums covers leeks, bulb onions, salad onions and garlic of which approximately 10,333 hectares were grown in 2017 (Defra, 2018a). With the exception of salad onions they are quite long season crops staying in the ground from four to seven months, making them more vulnerable to pest problems. Currently, there are no virus issues reported in alliums in the UK, though both *Onion yellow dwarf* virus (OYDV) and *Iris yellow spot virus* (IYSV) have been highlighted as key threats to the allium industry.

Onion yellow dwarf virus (OYDV)

OYDV was first reported in *Allium fistulosum* from Indonesia by Van Dijk and Sutarya (1992). Symtoms include bright systemic leaf striping, leaf distortion and stunting. OYDV in onion causes stem distortion, reduced number of flowers and seeds, and a loss of seed quality.

Iris yellow spot virus (IYSV)

IYSV was first reported on onion from Brazil in 1981 by de Avila *et al*., 1981. It was first discovered in the UK in June on a sample of Lisianthus (Eustoma) sent to the Central Science Laboratory. This is a potentially devastating disease that could seriously damage the onion and leek industries in the UK if it became established. IYSV symptoms in *Allium* spp. are yellow- to straw-coloured, diamond-shaped lesions on leaves and flowering scapes. As the disease progresses, the lesions coalesce, leading to lodging of the scapes. In seed crops, this could lead to a reduction in yield and quality. Early to mid-season infection in bulb crops results in reduced vigour and bulb size.

Table 33. Summary of viruses with the potential to infect alliums in the United Kingdom. Viruses highlighted in bold italics are discussed below.

Virus name	Acronym	Genus
Iris yellow spot virus	IYSV	Tospovirus
Leek yellow stripe	LYSV	Potyvirus
Onion mite-borne latent	Unknown	Potexvirus
Onion yellow dwarf	OYDV	Potyvirus
Pepper veinal mottle	PYMV	Potyvirus
Shallot latent	SLV	Carlavirus
Shallot mite-borne latent	Unknown	Potexvirus
Shallot yellow stripe	Unknown	Potyvirus
Sint-Jan's onion latent	SJOLV	Carlavirus
Tobacco rattle	TRV	Tobravirus
Welsh onion yellow stripe	Unknown	Potyvirus

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Vectors of key viruses

Table 34. Summary of known vectors and alternate host species of OYDV and IYSV.

Virus	Vector
OYDV	*M. persicae, Rhopalosiphum maidis, A. pisum*
IYSV	*Thrips tabaci, Frankliniella fusca Hinds*

Onion yellow dwarf virus (OYDV)

OYDV is transmitted by *M. persicae*, *R. maidis* and *A. pisum* in a non-persistent manner. The virus can also be transmitted by mechanical inoculation (Brunt *et al*., 1996).

Iris yellow spot virus (IYSV)

IYSV is transmitted by *Thrips tabaci* (onion thrips) and with less efficiency by *Frankliniella fusca Hinds* (tobacco thrips) in a persistent and propagative manner (Kritzman *et al*., 2001).

Alternate host species of key viruses

Table 35. Summary of known alternate host species of OYDV and IYSV.

Virus	Alternate host species
OYDV	*Chenopodium amaranticolor and C. quinoa*
IYSV	*Ameranthus retroflexus, Chenopodium album, Kochia scoparia, Lactuca serriola, Tribulus terrestris, Atriplex micrantha, Setaria viridis, Cichorium intybus, Arctium minus, Rumex cripus and Taraxacum officinale*

Onion yellow dwarf virus (OYDV)

Alternate hosts for OYDV include *Chenopodium amaranticolor* and *C. quinoa*. A current listing of the host range of OYDV is available at http:// www.alliumnet.org.

Iris yellow spot virus (IYSV)

IYSV has a relatively broad host range, including cultivated and wild onions, garlic, chives, leeks and several ornamentals (Table 35). Some weeds are naturally infected by IYSV and may serve as alternative hosts for the virus. A current listing of the host range of IYSV is available at http:// www.alliumnet.org.

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Detection and identification of viruses

Onion yellow dwarf virus (OYDV)

Virions of OYDV are filamentous and usually flexuous, at a size of about 750 nm. Fernández-Most recently, Tabanera *et al*., 2018 used a dot-blot assay and two-step RT-PCR to confirm the presence of OYDV in leek. The presence of OYDV was not apparently related to the symptomatology observed in the leeks since it was not detected in all symptomatic samples, and no specific symptomatology of OYDV was observed in these plants.

Tiberini *et al*., 2019 developed a RT-LAMP assay or detection of OYDV which was validated according to EPPO standard PM7/98 (2). This RT-LAMP assay has the potential to be used in laboratories with limited facilities and resources, as well as directly in the field..

Iris yellow spot virus (IYSV)

Virions of IYSV are pleomorphic of 80–120 nm in size (Kritzman *et al*., 2001). The genome of IYSV consists of trisegmented ssRNA. For immunodiagnosis, IYSV-specific polyclonal antibodies have been developed for ELISA testing and immunostrips are commercially available for rapid and accurate diagnosis (Pappu *et al*., 2006a), although some differences in the efficiency among the various commercial kits that utilize different polyclonal antisera have been reported (Tomassoli *et al*., 2009). For the detection of IYSV in individual thrips, a polyclonal antiserum has been developed to determine the seasonal dynamics of thrips transmitters in field-collected thrips populations (Bag *et al*., 2014). Molecular diagnostics based on 'traditional' PCR and real-time PCR for IYSV detection and diagnosis have been developed (Chingandu *et al*., 2012; Pappu *et al*., 2006) and genetic diversity studies on a global scale have been investigated by Iftikhar *et al*., 2014.

Management of viruses and vectors in the field

The central part of an integrated strategy for management of viruses in alliums is to grow virus-resistant or virus tolerant cultivars and to develop robust thrips management practices. Evaluation of cultivars and breeding material for virus resistance must be carried out under field conditions relying on natural disease pressure, as there is no reliable and efficient method for inoculation of onion by mechanical inoculation (Bag *et al*., 2015). Resistant varieties are not available, but a limited number of accessions with field tolerance have been identified (for example, see Diaz-Montano *et al*. 2010).

Integrated disease management tactics, including sanitation, crop rotation, thrips management, maintenance of optimal plant vigour, soil fertility, irrigation and physical

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separation of bulb and seed crops, can mitigate the effect of the disease. The effect of onion foliage colour on thrips feeding behaviour has been well documented: yellow–green coloured foliage is less attractive than blue–green-coloured foliage to thrips (Diaz-Montano *et al*., 2012; Jones *et al*., 1934; Molenaar, 1984). This trait is being exploited to develop onion cultivars showing less damage by thrips and IYSV. The use of straw mulch resulted in the reduction of thrips numbers by 30% (Schwartz *et al*., 2009). In a study in Utah, USA, it was found that reduced soil nitrogen in the absence of biostimulants can reduce onion thrips densities and final disease incidence without a significant loss in yield. It also created a favourable environment for soil microbial activity, and reduced the risk of leaching soil nitrate (Buckland *et al*., 2013).

The use of RNA interference (RNAi) by the expression of IYSV genomic sequences to generate transgenic plants with resistance to IYSV is an approach that could complement ongoing efforts to develop virus-resistant varieties using traditional plant breeding. However, efficient transformation and regeneration of onion continue to be a challenge. Transgenic onion expressing an RNAi construct was developed to reduce lachrymatory synthase activity, significantly reducing the level of tear-inducing lachrymatory factor (Eady *et al*., 2008), and this strategy could be further exploited to develop IYSV-resistant cultivars.

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Virus management in asparagus

In 2017, 2,470 hectares of asparagus were grown in the UK (Defra, 2018). Asparagus plantations are intended to be in the ground for at least 10 years, and in this time pests can build up if they are not managed effectively.

This review follows on from, and supplements the research conducted in AHDB Horticulture funded projects FV213, ‘’Asparagus: a literature search on viruses’’ and FV384/384a ‘’Asparagus: screening crops for virus infection’’. The resulting factsheet 02/13 ‘’Asparagus viruses’’ is also available for further reading.

Current viruses present in the UK

Table 36. Summary of viruses reported on asparagus in the United Kingdom, together with information on their mode of transmission and their vector. Viruses highlighted in bold italics are further discussed in the present section.

Virus	Acronym	Genus	Mode of transmission	Vector
Asparagus virus 1	AV-1	Potyvirus	Non persistent, aphid	*A. craccivora, M. persicae*
Asparagus virus 2	AV-2	Ilarivus	Persistent, mechanical	N/A
Tobacco streak virus	TSV	Ilarivus	Persistent, thrips, seed and pollen	*Frankliniella occidentalis and Thrips tabaci*
Cucumber Mosaic Virus	CMV	Cucumovirus	Non persistent, aphid, seed	More than 60 species of aphids

Asparagus virus 1 (AV-1)

AV-1 was first reported in asparagus from Germany by Hein, 1960. Asparagus infected with asparagus 1 and 2 viruses are significantly more susceptible to damage caused by *Fusarium oxysporum* f. sp. asparagi. AV-1 occurs naturally in asparagus plants that often are infected with TSV, AV-2 and CMV. Few, if any, symptoms are caused by AV-1 alone.

Asparagus virus 2 (AV-2)

AV-2 was first reported in asparagus from Denmark by Paludan, 1964. AV-2 is a member of the genus Ilarvirus and thought to induce the asparagus decline syndrome (Kawamura *et al*., 2014).

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New emerging viruses

Table 37. Viruses which are known to infect asparagus, but have not yet been identified in asparagus crops in the United Kingdom.

Virus	Acronym	Genus
Arabis mosaic virus	ArMV	Nepovirus
Tobacco ringspot virus	TRSV	Nepovirus
Tomato black ring virus	TBRV	Nepovirus
Strawberry latent ringspot virus	SLRSV	Nepovirus
Asparagus virus 3	AV-3	Potexvirus

Vectors of key viruses

Table 38. Summary of known vectors and alternate host species of AV-1 and AV-2

Virus	Vector
AV-1	*A. craccivora, M. persicae*
AV-2	No known vector

Asparagus virus 1 (AV-1)

AV-1 is transmitted by several aphid species in a non-persistent manner. The virus is also transmitted by mechanical inoculation but not by contact between plants and not in the seed or pollen.

Asparagus virus 2 (AV-2)

AV-2 is not transmitted by aphids and no insect vector has been reported. It is transmitted by mechanical inoculation and by grafting infected plant tissue onto a healthy plant. AV-2 is also transmitted in seed, by pollen to the seed and by pollen to the pollinated plant. The incidence of AV-2 in new plantings is directly correlated with the level of seed infection of AV-2 in seed lots used for planting. As a consequence, relatively low levels of AV-2-infected plants in or near fields where seed is harvested can result in significant levels of AV-2 contaminated seed. The experience of growers in the USA is that the use of virus-tested seed lots can virtually eliminate the occurrence of AV-2 in commercial asparagus fields and potentially make a significant contribution to the control of asparagus decline.

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Table 39. Summary of known alternate host species of AV-1 and AV-2.

Virus	Alternate host species
AV-1	*Allium tuberosum, Chenopodium spp., Gomphrena globose Tetragonia tetragonioides*
AV-2	*Beta vulgaris Chenopodium spp., Cucumis sativus, Glycine max, Gomphrena globose, Nicotiana spp., Ocimum basilicum, Petunia × hybrida, Phaseolus vulgaris, Vigna unguiculata*

Asparagus virus 1 (AV-1)

AV-1 has a relatively limited host range but there are several susceptible host species other than asparagus, some of which are important weed species (e.g. *Chenopodium* spp.).

Asparagus virus 2 (AV-2)

AV-2 has a relatively wide host range compared with AV-1. Details of alternate host species are provided in Table 39.

Detection and Identification of Viruses

The three principal viruses associated with asparagus decline can be readily detected using ELISA. These viruses can also be detected by host range studies but these are very time-consuming and are not suitable for differentiation of some viruses in mixed infections.

Asparagus virus 1 (AV-1)

Leaf sap containing AV-1 contains few virions. Virions present are filamentous and non-enveloped, with a clear modal length of 740 nm and 13 nm wide. The genome of AV-1 consists of ssRNA. Replication does not depend on a helper virus. Blockus *et al*., 2014 determined the complete genome sequences of two AV-1 isolates differing in their ability to cause systemic infection in *N. benthamiana*. The closest relatives of AV-1 in amino acid sequence identity were plum pox virus (54 %) and turnip mosaic virus (53 %), corroborating the classification of AV-1 as a member of a distinct species in the genus Potyvirus. Tiberini *et al*., 2014 developed a RT-PCR assay for simultaneous detection of AV-1 and AV-2 in asparagus crops in Italy.

Asparagus virus 2 (AV-2)

Virions of AV-2 are isometric and non-enveloped. Kawamura *et al*., 2014 performed sequence analyses on AV-2 isolates to determine whether pollen transmission could result in mixed infections. Shimura *et al*., 2013 cloned the coat protein (CP) and the 2b protein (2b) genes

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from a range of AV-2 isolates and found that the sequence for CP and for 2b was highly conserved, suggesting that AV-2 from around the world is almost identical.

Novel technologies

Transformation in asparagus has been demonstrated as early as in the '90 with a range of approaches, including Agrobacterium, protoplast electroporation, and biolistics (Klocke *et al*., 2010) but regeneration and transformation protocols have not been developed further. Knowledge on Asparagaceae genomes is scarce compared to e.g. the Solanaceae and Cucurbitaceae. Genomic resources for asparagus are limited because of their large, extremely complex, repetitive, and often polyploid genomes and long generation times.

Management of viruses and vectors in the field

For perennial vegetable crops such as asparagus, a clean start is of particular importance as there are limited opportunities for pest, disease and weed control. Control of AV-2 is key to control of viruses in asparagus, and therefore the virus component of asparagus decline. AV-2 is seed-transmitted and the level of AV-2 in new planting is correlated with levels of virus infection in the seed so it is crucial that seed is virus-tested and only seed lots that are free of virus are grown.

The virus status of asparagus seed imported into the UK for commercial use is unknown. However, it is known that seed is currently not tested for the presence of virus and neither are plants that are used for seed production. AV-2 is present in the embryo of the seed so cannot be removed by surface sterilisation of the seed. Heat treatment of seed above 32°C is not an option for virus eradication as asparagus seed is rendered non-viable above 31°C. Disinfecting cutting tools between plants may help to control the spread of AV-1 and AV-2 but due to the intensive nature of asparagus harvesting this is not practical or economic (McPherson *et al*. 1998, 2011).

Once new virus-free plantations have been established it is important to keep them virus-free by segregating them from older, virus-infected plantings, disinfecting cutting tools between plantations or having a dedicated set of cutting tools for new plantations and disinfecting cultivating machinery between plantations. Chemical control of aphids is based around a small number of active substances including lambda-cyhalothrin and spinosad. Chemical control is costly, environmentally sensitive and would have minimal benefits, as it would not control AV-2. An alternative approach is use a cross-protection approach against AV-2 using a mild strain of the virus to protect against more severe strains. Other possibilities for long-term control are to produce virus-free clonal material by meristem tip culture and maintain it in isolated fields away from asparagus production (McPherson *et al*., 1998, 2011).

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If not controlled in the early years, weeds which can act as alternate host for viruses can build up very quickly. Once the crop is established, weed is control is targeted by a range of approaches including herbicides pre- and post-harvest, hand rogueing, and overwinter living mulches between the rows. Intercropping or undersowing with a manageable species could suppress weeds in the non-competitive crops.

Breeding for aphid resistance and host resistance and/or tolerance

Lohwasser and Börner (2018) report on the maintenance, taxonomy and availability of plant genetic resources of asparagus. Only 1,284 samples of 63 Asparagus species are maintained with the largest asparagus collections in France (INRA) and the USDA-ARS. The gene pool of asparagus is very well known but the accessions are not taxonomically described, hence their use for breeding is very difficult. Npthnagel *et al*., 2017 evaluated forty-four cultivars, gene bank accessions and breeding lines of asparagus as well as thirty-four accessions of wild relatives of Asparagus for resistance to AV-1 using natural and artificial inoculation methods. AV-1 infections were verified by DAS-ELISA and RT-PCR approaches. All tested plants of *A. officinalis* germplasm were susceptible to AV-1 infection. In contrast, in 276 plants of 29 Asparagus wild accessions, no virus infection could be detected.

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Virus management in protected tomato

Tomato is one of the highest value crops grown in the UK, with 65,000 tonnes of fruit produced in over 170 ha of protected glass (Defra 2017). The majority of commercial tomato production in the UK is grown in rockwool or coir substrate, with some organic crops produced in certified soil. Growing in protected structures enables precise crop steering, maximising fruit quality and yields, whilst limiting pest and disease issues which may occur in the field. However, pest and disease issues can rapidly develop in these densely populated areas, placing the crop at risk.

Over 100 viruses naturally infect tomato (*Solanum lycopersicum*), with more demonstrated to infect via artificial inoculation (Plant Virus Online). Symptoms differ by virus species and strain, with individual strains exhibiting differences in symptom expression and severity. Tomato plants can also become infected by multiple viruses, which can exacerbate symptoms (Luria, Smith *et al*. 2017). Tomato viruses can affect all parts of the host plant. The roots, stems, foliage and fruit. Infection usually leads to a reduction in fruit quality and/or fruit number, impacting the quantity of marketable fruit, leading to economic loses.

UK tomato production is predominantly an annual crop with plant material removed from site structures at the end of each year. This is followed by cleaning and complete disinfection before new plants are brought in, mainly sourced from UK or Dutch propagators. Some sites, however may limit their turnaround sanitation to allow natural populations of predators, for instance, to build up earlier in the season. Comprehensive site clean-up allows for the elimination of pest and disease persistence, with many viral outbreaks eradicated in this way. There is an increasing trend to plant overwintering lit crops that may or may not be interplanted – interplanted crops have less or even no opportunity to clean up between crops.

Current viruses present in the UK

Many species of virus, from many genera infect tomato, including potexvirus, tospovirus, crinivirus, begomovirus and tobamovirus spp. The recent main viral threats to UK commercial tomato production are listed in Table 33. This includes viruses which are currently present in the UK and those which have occurred in the past, but are no longer present (e.g. due to the widespread use of varieties with durable genetic resistance).

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Table 33. Summary of the current, or recent, viral threats to UK tomato production.

Virus name	Acronym	Genus	Reference
Pepino mosaic virus	PepMV	Potexvirus	(Vlugt, Stijger *et al*. 2000)
Tomato spotted wilt virus	TSWV	Tospovirus	(O'Neill and Bennison 2010)
Tomato mosaic virus *Tobacco mosaic virus*	ToMV TMV	Tobamovirus Tobamovirus	(Broadbent 1976) (Milinkó 1966)

Pepino Mosaic Virus (PepMV)

PepMV, a potexevirus, is one of the most economically important tomato viruses worldwide (Novak, Milanović *et al*. 2011) and is the most common viral issue currently affecting UK tomato production. The virus is responsible for severe economic losses estimated to average £70, 000 per production site (PC 181, 2001). Originally identified in Peru in 1974, PepMV was found in the Netherlands in 2000 (Vlugt, Stijger *et al*. 2000), the virus is highly infectious and control options are limited.

Tomato spotted wilt virus (TSWV)

Tomato spotted wilt virus, TSWV, is a tospovirus affecting over a thousand ornamental and edible crop species, including tomato (Srinivasan, Joseph *et al*. 2011). First described in the UK in 1931, a time where it was much more common on tomato, there have been no recent outbreaks of TSWV on tomato reported. Outbreaks on sweet pepper occurred during 2008 and 2009 (O'Neill and Bennison 2010). TSWV has a wide host range and is responsible for large reductions and crops losses of up to 100% (Roselló, José Díez *et al*. 1996). Annual losses have been estimated at over $1 billion in open field and greenhouse grown crops worldwide (Karavina and Gubba 2017). The disease is controlled well through the cultivation, use of resistant varieties and management of the principle vector, WFT (*Frankliniella occidentalis*).

Tomato mosaic virus (ToMV) and Tobacco mosaic virus (TMV)

ToMV and TMV are both tobamovirus and are present worldwide in field and protected crops. Historically much more common, ToMV incidence has decreased with the development and use of resistant varieties. TMV is typically restricted to tobacco, but does occasionally infect tomato. ToMV in tomato has been well controlled for many years by incorporation of the Tm^2 gene.

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New and emerging viruses

Over 100 tomato virus species exist, which are causing significant problems overseas, but which do not currently occur in the UK. These include viruses which are endemic to the EU and/or further afield, or have occurred in the UK, but have since been eradicated.

Table 34. Summary of potentially emerging viruses to UK tomato production.

Virus name	Acronym	Genus
Tomato chlorosis virus	ToCV	Crinivirus
Tomato infectious chlorosis virus	TICV	Crinivirus
Tomato brown rugose fruit virus	ToBRFV	Tobamovirus
Southern tomato virus	STV	Amalgavirus
Tomato yellow leaf curl virus	**TYLCV**	**Begomovirus**

Tomato yellow leaf curl virus is not currently present in the UK, and is currently deemed low risk, and has not been covered by this review.

Tomato chlorosis virus (ToCV) and Tomato infectious chlorosis virus (TICV)

Tomato chlorosis virus (ToCV) and *Tomato infectious chlorosis virus* (TICV) are both whitefly vectored crinivirus species of the family, *Closteroviridae*. ToCV was first identified in 1993 in California and TICV in Florida in 1998. Since this time these diseases have been identified in Europe, Asia, South America and Africa. ToCV was first described in the Netherlands in 2017, and was later identified on eleven tomato production sites. No varietal resistance is available in commercial tomato varieties.

Tomato brown rugose fruit virus (ToBRFV)

ToBRFV is a newly emerging tobamovirus threat to tomato and sweet pepper production worldwide. Similar to other tobamoviruses, ToMV and TMV, the virus is transmitted mechanically. Unlike these viruses no varietal resistance to ToBRFV currently exists. ToBRFV was identified in Jordan in 2015 (Salem, Mansour *et al*. 2015), but was found to have affected crops in Israel during 2014 (Luria, Smith *et al*. 2017). More recent disease outbreaks have occurred in Germany (Menzel, Knierim *et al*. 2019), Italy (EPPO 2019), Mexico (Cambron-Crisantos, Valencia-Luna *et al*. 2018), the USA (California), Turkey, and China. The scattered geographical distribution, absence of a species-specific diagnostic test and the similar symptoms to other viral diseases, e.g. PepMV, suggests that ToBRFV may occur in other locations, but has not been correctly identified. The first case of ToBRFV in a UK commercial crop was reported on July 12th 2019. At the time of writing of this review the

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source of this outbreak is currently unknown. The risk of infection to other sites remains high – especially where production sites handle imported fruit.

Southern tomato virus (STV)

STV is emerging tomato virus belonging to the new genus *Amalgavirus*. Originally identified in North America, the disease has a worldwide distribution and has been detected in several EU countries including, France (Candresse, Marais *et al*. 2013), Italy (Iacono G, Hernandez-Llopis D *et al*. 2015) and The Canary Islands, Spain (Verbeek, Dullemans *et al.* 2015).

STV was first identified in the UK in April 2019 and has been confirmed on three UK sites. STV symptoms are similar to PepMV/ToBRFV, but are not as severe, and STV is not believed to be an economically damaging disease. Misdiagnosis of STV likely means the disease may be present on more sites, but has not yet been recognised. Unlike PepMV and ToBRFV, STV is not easily mechanically transmitted (or transmitted by grafting), and is currently believed to have entered the UK via infected seed, (not yet confirmed).

Vectors of key viruses

The warm, protected environment of the glasshouse provides optimal conditions for pest numbers to rapidly increase, enabling virus transmission to a large number of plants over a short time period. Preventing introduction of insect vectors, including thrips, aphids and whitefly, is critical to controlling insect vectored diseases. Once pests are found, focus should be directed at eliminating, or reducing pest numbers. Some virus diseases of tomato are transmitted by species of aphids e.g. *potato virus Y* (PVY), or nematode e.g. *Tomato ringspot virus* (ToRSV). Outbreaks of such diseases in the UK have been relatively rare. Beneficial insects, including bees and predators, including *Macrolophus*, have been implicated with the transmission of tomato viruses, including some tobamoviruses. These insects are critical to pollination/pest control and this issue insurmountable.

Virus-free and high health plant material should be sourced from clean propagators, using disease-free seed. For mechanically transmissible viruses, e.g. tobamoviruses, on-site hygiene, including equipment/machinery and workers/visitors is the main control strategy for preventing spread once a virus is established on site. If visitors don’t actually need to see the crop then deny them entry.

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Table 35. Vectors and transmission routes of the key tomato viruses that threaten UK crops.

Virus	Vector/transmission route
Pepino mosaic virus	Seed-borne (low rate, not PE strain). Infected plant material (leaf to leaf contact), including from debris. Mechanical transmission, hands, clothing, knives (grafting), equipment etc. Bumblebees and other insects, including certain biologicals – *Macrolophus.* Hand pollination. Nutrient solution (greater risk in circulated systems). Infected soil (organic crops). Air flow.
Tomato spotted wilt virus	Thrips – Western flower thrips, *Frankliniella. occidentalis and* Tobacco thrips, *Frankiniella. fusca.*
Tomato chlorosis virus	Whitefly species - Predominantly, but not limited to *Bemisia tabaci*.
Tomato infectious chlorosis virus	Whitefly species - Predominantly, but not limited to *B. tabaci*.
Tomato mosaic virus	Seed-borne. Infested plant material (including leaf to leaf contact). Mechanical transmission, hands, clothing, knives (grafting), equipment etc. Hand pollination. In nutrient solution (greater risk in circulated systems). Infested soil (organic crops).
Tobacco mosaic virus	Infested plant material (including leaf to leaf contact). Mechanical transmission, hands, clothing, knives (grafting), equipment etc. Hand pollination. In nutrient solution (greater risk in circulated systems). Infested soil (organic crops).
**Tomato brown rugose fruit virus*	Seed-borne (likely) Infested plant material (leaf to leaf contact), including from debris. Mechanical transmission, hands, clothing, knives (grafting), equipment etc. Bumblebees and other insects, including biologicals – *Macrolophus.* Hand pollination. Air flow.
Southern tomato virus	Seed-borne (high rate >70%)

*As a consequence of the recent emergence of ToBRFV there is currently no information in the literature regarding the spread of ToBRFV via infested soil or via nutrient solution. These routes of transmission are considered likely. Infested seed has been identified, sourced from Peru and an EU member state, but seed-borne transmission has not been demonstrated.

Pepino mosaic virus (PepMV)

High concentrations of PepMV accumulate in fruit and leaves, and PepMV is spread easily through mechanical contact, without the need for an insect vector (Mayne and O'Neill 2016). Bumblebees have been demonstrated to mechanically transmit PepMV during pollination,

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and between bumblebees within hives (Shipp, Buitenhuis *et al*. 2008). The virus is seed-borne and there is evidence that it may spread through the nutrient feed (Schwarz, Beuch *et al.* 2010). The ability of PepMV to be transmitted mechanically, rather than reliant upon insect vector species, increases the risk of spread between sites. Site visitors, including agronomists, reps, maintenance workers etc., and equipment e.g. trays, machinery, trolleys, are all potential vectors if appropriate disinfection and biosecurity measures are not followed.

Tomato spotted wilt virus (TSWV)

TSWV is transmitted via an insect vector, predominantly adult Western Flower Thrips (WFT), *Frankliniella occidentalis* (E. Ullman 1992). The number and frequency of TSWV outbreaks increased following the establishment of WFT to the UK in 1986.

Only thrips which feed on infected plants at the nymph stage are able to transmit the virus as adults, however these adults retain the virus permanently, enabling them to continue to infect plants during feeding (de Assis Filho, Deom *et al*. 2004). As a consequence, small populations of WFT are sufficient to cause extensive infection within crops and WFT control measures are needed. The import and transport of virus-infected plants to sites is also responsible for TSWV spread.

Tomato mosaic virus (ToMV) and Tobacco mosaic virus (TMV)

Tobamoviruses are transmitted via contact, hands, knives, equipment etc. As a consequence, disease distribution frequently occurs down rows of crops. Although not transmitted by invertebrate vectors, e.g. thrips and whitefly, pollinating bumblebees have been demonstrated to transmit TMV (Okada, Kusakari *et al*. 2000). These viruses are seed-borne, present both within and on the coating of seed. ToMV is very stable and able to persist in plant debris for at least six months (Mehle, Gutiérrez-Aguirre *et al*. 2018). ToMV virus has been shown to survive in the soil and substrates for several years.

Tomato chlorosis virus (ToCV) and Tomato infectious chlorosis virus (TICV)

ToCV is vectored exclusively by the Sweet potato whitefly (a.k.a. Silverleaf whitefly, *Bemisia tabaci*), whereas TICV may be spread by several species of whitefly, including *B. tabaci*. The virus is injected directly into the phloem stream via the whitefly stylet, and transmission can occur within as little as one hour of feeding. As a consequence of this transmission route, the virus is unable to be transmitted by mechanical means, restricting its spread compared to other viruses, including PepMV. TICV is able to remain viable for three to five days within the adult whitefly, allowing individuals to quickly infect multiple plants (Fox and Buxton-Kirk 2017).

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Tomato brown rugose fruit virus (ToBRFV)

Similar to other tobamoviruses, ToBRFV is transmitted through contact with infected plant material and mechanical means. Transmission in this manner, before the virus was identified, enabled the rapid spread of ToBRFV through much of Israel during 2014 (Salem, Mansour *et al*. 2015). Situating a bumblebee hive collected from a ToBRFV contaminated greenhouse to a house with uninfected plants spreads ToBRFV disease, demonstrating bumblebees also act as vectors (Levitzky, Smith *et al*. 2019). At this stage it is unclear if the bees mechanically transmit the virus, or it is a result of the transmission of infested pollen grains onto the host plant stigma. The virus is likely to be seed-borne and is expected to remain active for several months on the surface, or within the seed endosperm. ToBRFV is also able to survive in plant debris for several months.

Southern tomato virus (STV)

Information on STV transmission is scarce. The virus is transmitted at a high rate (>70%) in seed, with the virus accumulating to a high level in the seed germplasm, and the distribution of infected seed is viewed as the key means of spread. STV is not easily transmitted by mechanical transmission routes or through grafting (Sabanadzovic, Valverde *et al.* 2009) and transmission by insect vectors is currently unknown.

Alternate host species of key viruses

Several weed and ornamental species act as natural reservoirs for the key tomato viruses The tobamoviruses, (ToMV, TMV and TBRFV), as well as PepMV, are stable outside of the hosts, transmitted to tomato via direct contact with virus infested plant tissue. Viruses spread by virulferous vectors, such as whitefly (ToCV, TICV) and thrips (TSWV) feed on infested alternate host species and transfer viral particles to uninfected tomato. Several alternate host species demonstrate asymptomatic infection, e.g. ToBRFV in petunia. Many alternate host species of the key tomato viruses, including ToBRFV, may therefore remain unknown.

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Table 36. Alternate host species for the key tomato viruses

Virus	Alternate host
Pepino mosaic virus	Solanaceae hosts - *Lycopersicum chilense, L. chmielewskii, L. parviflorum, L. peruvianum* Weed hosts – *Amaranthus sp., Malva parviflora, Solanum. nigrum, Sonchus oleraceus*
Tomato spotted wilt virus	*Amaranthus* spp*., Oxalis corniculata, Poa annua, Sonchus* spp*., Veronica* spp**.** Very large host range: over 86 botanical families
Tomato chlorosis virus	*Solanum tspp, Capsicum annuum, Physalis spp., Zinnia elgans*
Tomato infectious chlorosis virus	*S. tuberosum, Petunia spp. Chenopodium spp., Picrisechloides, Senecio spp.*
Tomato mosaic virus	*Capsicum spp., Nicotiana tabacum, Petunia spp., S. tuberosum*
Tobacco mosaic virus	At least 39 solanaceous species including, *C. annuum, S. melongena, S. tuberosum, S. torvum, S. nigrum.* Wide host range, affecting over 40 dicotyledonous botanical families
Tomato brown rugose fruit virus	*Capsicum annuum, Solanum spp. Chenopodium spp., Petunia hybrid, Nicotiana tabacum,*
Southern tomato virus	No information on alternate hosts of STV were found in the literature

This table shows the most common alternate host species for the key viruses only and should not be considered exhaustive

Detection and identification of viruses

Tomatoes are high value, continually fruiting vine crops. Crops are walked and monitored frequently by growers/site staff allowing pest and disease issues to be rapidly identified. However, viral symptoms vary based on strain, growth stage, growing environment and tomato variety, and can easily be mistaken for nutritional deficiencies or an alternate disease/disorder.

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Table 37. Currently available detection techniques for the key viruses of tomato.

Virus	Detection method
Pepino mosaic virus, (PepMV)	ELISA RT-PCR. Lateral flow devices. Electron microscopy. Immuno-electron microscopy. Immunoblotting
Tomato spotted wilt virus (TSWV)	ELISA (infested tissue and vector). RT-PCR. Transmission test – *Petunia hybrid*. Density in solvent solution. Lateral flow devices Electron microscopy. Immuno-electron microscopy. Immunoblotting
Tomato mosaic virus (ToMV) *Tobacco mosaic virus* (TMV)	ELISA (infested tissue and vector). RT-PCR. Transmission tests – *C. quinoa*, *N. glutinosa*. Density in solvent solution. Lateral flow devices. Electron microscopy. Immunoblotting
Tomato chlorosis virus (ToCV) *Tomato infectious chlorosis virus* (TICV)	ELISA (infested tissue and vector). RT-PCR. Electron microscopy Immunoblotting
Tomato brown rugose fruit virus (ToBRFV)	ELISA . RT-PCR. Transmission test – sweet pepper. Electron microscopy. Immunoblotting
Southern tomato virus	RT-PCR (including RT-LAMP). Immunoblotting

Electron microscopy will identify a virus to the genus level, but should be combined with an alternate test e.g. ELISA/RT-PCR for exact diagnosis. Alternatively Immuno-electron microscopy should be used where antisera is available.

Pepino mosaic virus (PepMV)

Early symptoms develop as yellow mosaic spotting on leaves which develop into severe chlorosis in heavily infected tissue. Marbling, from uneven ripening develops reducing the marketability of tomato fruit, which also affects quality (Hanssen and Thomma 2010). PepMV is a mechanically spread potexvirus and this transmission route is well documented, including by biologicals e.g. Macrolophus and pollinating bumblebees (Shipp, Buitenhuis *et al*. 2008).*Nicotiana occidentalis* 37B is a useful indicator plant for PepMV sap transmission tests since it reacts with a different symptomatiology to each of the PepMV strains, EU, CH2 and US1 (Blystad, Vlugt *et al*. 2015).

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Detection and Identification Methods based on Viral Coat Protein

Mumford *et al*., 2001 sequenced the PepMV viral coat protein of 14 European isolates and one Peruvian isolate. European isolates showed over 99% genetically similarity, but were only 96-97% similar to the Peruvian isolate. The weight of the major constituent of the virus coat protein was identified as 20 kD (Zilahi-Balogh, Shipp *et al*. 2007), Lateral flow devices, developed for PepMV detection are available from several manufacturers e.g. Lynchwood diagnostics. These tests work on a qualitative basis and rapidly detect antigens specific to the virus in question, providing a positive/negative result in minutes. PepMV is a non-enveloped filamentous virion, 10 nm wide, with a modal length of 512 nm (plant virus online). Transmission electron microscopy (TEM) is used to identify potexviruses, but is unable to differentiate between closely related types, e.g. PepMV, *Potato aucuba mosaic virus* (PAMV) and *Potato virus X* (PVX). Immuno-electron microscopy (IEM) with PepMV antiserum can detect PepMV. Vlught, R., *et al.* used IEM to differentiate PepMV from PAMV and PVX, both of which infect tomato. Hasiów-Jaroszewska, *et al.* 2011 used western blotting to identify and compare the accumulation of PepMV in infected leaves using an antibodies against PepMV. This method demonstrated comparable accumulation of PepMV in plants infected with two different strains of the virus.

Tomato spotted wilt virus (TSWV)

Symptoms vary, with yellow mottling and line patterning, which eventually develops to dark black rings, turning to necrotic lesions, with lesions also developing on petioles and stems. Young leaves become deformed, curling inwards and bronzing occurs, predominantly on the upper surfaces of young leaves. Fruit set is reduced in infected plants and fruit develop yellow spots turning orange through to brown (Roselló, José Díez *et al*. 1996). TSWV is capable of killing seedlings and reduces the vigour of more established plants, leading to stunting and the curving of the stem (O'Neill 2008). Sap-transmission tests using diagnostic species, including *Petunia hybrid*, gloxinia and globe amaranth rapidly identify (within 2-4 days) the presence of TSWV (Allen & Matteoni, 1991). Petunia is not systemically infected with TSWV and affected plants will not represent a significant inoculum source for existing tomato crops within the same compartment. Sap-transmission is most efficient in younger plants, whilst transmission is reduced from older infected plants (EPPO data sheet on quarantine pests (*tomato spotted wilt virus*)). One sedimenting component in purified preparations; sedimentation coefficient 550 s. Density 1.21 g cm^{-3} in sucrose. (Plant Virus Online)

Detection and Identification Methods based on Viral Coat Protein

ELISA using both monoclonal and polyclonal antibodies to nucleocapsid proteins, and glycoproteins positively identifies different isolates of TSWV, as well as detects defective

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forms (Avila, , *et al.* 1990). Greater variation was detected in TSWV nucleocapsid proteins than glycoproteins suggesting these are a more suitable target for identifying different viral strains. Lateral flow devices, developed for TSWV detection are available from several manufacturers e.g. Lynchwood diagnostics, Adgen Phytodiagnostics. These tests work on a qualitative basis and detect antigens specific to the virus in question, rapidly providing a positive/negative result.

TSWV virions particles are enveloped, spherical and 70-110 nm (modal 85 nm) in diameter, containing an internal nucleoplasmid protein, two membrane proteins and a large protein. Electron microscopy is a reliable method for the identification of TSWV, however this process is time consuming and requires fixation of the particles to prevent distortion. Immune-electron microscopy is available for detecting TSWV and has been used by Kiajima, E. *et al.* 1992, using gold labelled protein A and antibodies prepared against virus particles and nucleocapsids. Dot immunobinding assays (DIBA) using riboprobes to TSWV was shown by Huguenot*, et al.,* 1990, to detect several TSWV isolates in several host species and was as effective as ELISA.

***Tomato mosaic virus* (ToMV) and *Tobacco mosaic virus* (TMV)**

Symptoms vary by strain, variety and growth stage and include leaf distortion, the development of chlorotic mosaic symptoms on leaves and the swelling of the first truss. Fruit set is reduced and the quality of set fruit is impacted with the development of bronzing (O'Neill, Raymond *et al*. 2011). Both viruses are tobamoviruses and demonstrated to be spread via mechanical contact. Several susceptible host species are used for diagnostic purposes with systemic mosaic symptoms occurring in *Nicotiana tabacum* and local lesions developing in *Chenopodium quinoa* and *N. glutinosa* infected with TMV. *Phaseolus vulgaris* cv. Black Turtle 1 is also used to identify and differentiate between ToMV and TMV (Fillhart, Bachand *et al.* 1998). Sedimentation coefficient 194 s. Density 1.325 g cm^{-3} in CsCl. Isoelectric point pH 3.5.

Detection and identification methods based on Viral Coat Protein

ELISA tests using antisera to TMV and coat proteins is used to directly detect and differentiate between strains of TMV (van Regenmortel and Burckard 1980). Similar ELISA techniques based on coat protein assays can be used to detect ToMV. Precipitation and agglutination tests, including lateral flow devices are available for the rapid on-site detection of both TMV and ToMV

Microscopy - immunoelectron and electron

Both viruses are similar in physical structure, rod shaped, straight, and unenveloped with length of 300 nm and a width of 18 nm. As a consequence of this structural homogeneity,

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microscopy alone should be used in combination with another diagnostic technique, e.g. ELISA, for exact species identification.

Immunoblotting

Immunoblotting techniques can be used to identify both TMV and ToMV. Improvements to assays, have enabled enhancements, such as modifications to dot immunobinding assays (DIBA) to rapidly detect low levels of virus presence using a reduced quantity of antibody (Hibi and Saito 1985).

Tomato chlorosis virus **(ToCV) and** ***Tomato infectious chlorosis virus*** **(TICV)**

ToCV and TICV infections causes extensive crop damage, significantly reducing yields. Both diseases are identified via the development of leaf chlorosis, however this is easily misdiagnosed as nutritional deficiency or phytotoxic damage (Fox and Buxton-Kirk 2017). Symptoms develop two to three weeks after infection, as mottling on the lower leaves. Symptoms increase towards the growing point with leaves thickening, becoming brittle and eventually rolling. Interveining symptoms develop on older leaves which undergo bronzing and die. ToCV and TICV are phloem transmitted by whitefly. Artificial inoculation of indicator plants, including *N. benthamiana* and *N. clevelandii,* differentiates between ToCV and TICV, with TICV infection alone leading to necrotic flecking (Wisler, *Li et al*. 1998). Single leaf grafting of ToCV infested leaflets led to systemic infection in 88% of test plants in the absence of the vector (Lee, Kim *et al*. 2017), demonstrating some ability of the virus to be transmitted mechanically.

Detection and Identification Methods based on Viral Coat Protein

DAS-ELISA using polyclonal antisera to capsid proteins are used to identify ToCV and TICV A triplex RT-PCR assays is also available to detect the presence of ToCV and/or TICV in one step (Jacquemond, Verdin *et al*. 2009).

Microscopy – immunoelectron and electron

ToCV and TICV particles are flexuous and filamentous, with modal lengths of 800-850 nm long and 12 nm wide (EPPO Tomato Infectious chlorosis virus). Microscopy will identify the presence of these criniviruses, however ELISA and RT-PCR should be used for exact identification.

Immunoblotting

Dot-blot hybridisation probes with ToCV and TICV specific primers are used for reliable identification (EPPO Tomato chlorosis crinivirus, EPPO).

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Tomato brown rugose fruit virus (ToBRFV)

ToBRFV is a newly emerging virus and at the time of writing, literature on this disease was limited. ToBRFV symptoms are similar to, and easily mistaken for alternative viral infections, including PepMV and TMV. Symptoms vary by cultivar, growth stage etc., but the virus can affect an entire crop. Chlorosis, development of mosaic and mottling features occur on leaves, along with occasional leaf narrowing and necrotic spots can develop on peduncles, calyces and petioles(Luria, Smith *et al*. 2017). Fruit quality is severely reduced, with yellow/brown rugose symptoms developing, resulting in non-marketable deformed fruit suffering uneven maturation. ToBRFV is mechanically spread and also affects sweet pepper, posing a heightened risk at sites where both crops are grown. Seed transmission of tobamoviruses is common, but ToBRFV transmission in this manner has not yet been confirmed (but is considered likely). Two batches of seed, one from Peru, another from an EU state, were positively identified as being infested and bumblebees have been demonstrated to transmit the virus during pollination (Levitzky, Smith *et al.* 2019). Petunia is an asymptomatic host and eggplant is not a host (Luria, Smith *et al.* 2017).

Detection and identification methods based on Viral Coat Protein

No information on the physical properties of ToBRFV specific to viral coat proteins was found in the literature, however ELISA and RT-PCR identification techniques using ToBRFV coast proteins will likely be developed in the future. No precipitation or agglutination tests are known to have been developed for the rapid detection of ToBRFV, however this is likely in development.

Microscopy - immunoelectron and electron

Transmission electron microscopy (TEM) of ToBRFV reveal a tabamovirus particle size of 235±123 nm in length and 18 nm wide. As with all tobamoviruses electron microscopy is insufficient alone for positive identification of ToBRFV and this should be combined with ELISA or RT-PCR.

Immunoblotting

Luria *et al*., 2017 used western blotting to positively detect ToBRFV.

Southern tomato virus (STV)

STV is an emerging virus in UK crops and at the time of writing, literature on this disease was limited. STV symptoms are easily mistaken for those of other virus infections, including PepMV and ToBRFV, and include stunting, discolouration and a reduction in fruit size (Puchades, Carpino *et al*. 2017). Unlike PepMV/ToBRFV, STV symptoms are not very damaging and the virus is repeatedly detected in asymptomatic plants (Alcala-Briseño,

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Coskan *et al*. 2017). Therefore, infection with STV alone, may be insufficient to cause visible symptoms, raising its pathogenic nature into question. Mixed infections, with other viruses may be necessary for symptom development, but the role of STV in mixed infections is unknown (Elvira González, Carpino *et al*. 2018). STV is transmitted poorly through mechanical contact and grafting, but is spread via infected seed. No indicator plants, or insect vectors, are known to exist.

Detection and identification methods based on Viral Coat Protein

A molecular diagnostic test developed by Puchades *et al*., 2017 using a digoxigenin-labelled RNA probe to the STV putative viral coat protein is sensitive enough to rapidly detect viral infection from sap extracts.

Detection and Identification Methods based on Viral Nucleic Acid

Pepino mosaic virus (PepMV)

Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using commercial antisera is used to qualitatively identify PepMV presence in infected plants and seeds. Real-time reverse transcription PCR (RT-PCR) and immunocapture reverse transcription are also used to identify PepMV infection. These techniques can be used for strain differentiation, such as multiplex RT-PCR formats and RT-PCR in combination with restriction fragment length polymorphism or DNA sequencing (PM 7/113 (1)). ELISA and RT-PCR are the recommended diagnostic tests for all viruses covered in this review.

Tomato spotted wilt virus (TSWV)

ELISA, using extracts from both infected plants and virulferous thrips is the most common serological test for detecting TSWV (Bandla *et al*, 1994). Polyclonal antibodies to the entire virion using DAS-ELISA, rather than structural proteins is recommended for the detection of different isolates of TSWV (Adam *et al.* 1991, Wang & Gonsalves 1990). Detection via PCR, originally developed by Mumford *et al.* (1994), has been improved with real time RT-PCR now available for detection of single and bulked leaf samples (Dietzgen, R. *et al.* 2005). This technique proved more sensitive than DAS-ELISA in detecting TSWV in bulked samples.

Tomato mosaic virus (ToMV) and Tobacco mosaic virus (TMV)

ELISA using extracts from plants and virulferous thrips is the most common serological test used for detecting ToMV and TMV. RT-PCR is also used to identify infection with these two viruses. Kumar, 2011 *et al.,* 2011, developed a highly sensitive multiplex PCR test which can rapidly detect and differentiate singular or simultaneous infection with TMV and ToMV.

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Tomato chlorosis virus (ToCV) and Tomato infectious chlorosis virus (TICV)

DAS-ELISA assays using polyclonal antisera are available for the identification of ToCV and TICV, allowing rapid identification and differentiation of these two closely related viruses (Duffus, Liu *et al*. 1996). RT-PCR is also used for identifying both ToCV and TICV.

Tomato brown rugose fruit virus (ToBRFV)

ELISA and RT-PCR assays have been developed for ToBRFV detection. (*Tomato brown rugose fruit virus* (EPPO), (Luria, Smith *et al*. 2017). Antisera is available, but cross-reactivity with other tobamoviruses, including TMV is known to occur. Sequencing is recommended to ensure the exact species is identified. Incorrect identification of ToBRFV as TMV may explain the scattered worldwide geographical distribution of the disease.

Southern tomato virus (STV)

Molecular techniques, including RT-PCR and RT-LAMP assays are used to detect the presence of STV infection in tomato, as antibodies against STV are not available. Sensitive real-time quantitative RT-PCR has been developed which can quantify viral load in different plant tissues, as well as monitor viral accumulation over time (Elvira González, Carpino *et al*. 2018).

Novel technologies

***Pepino mosaic virus* (*PepMV*)**

Non-destructive seed testing for seed-borne viruses

Traditional seed treatment, sodium hypochlorite (1-3%) is not always sufficient to control seed-borne infection, especially where the virus is present within the endosperm. Destructive tests, such as ELISA, are available, but typically require several hundred seeds, which can be prohibitively expensive (commercial tomato seeds can cost in excess of 50 pence each). A ‘wash and grow’ seed testing technique was developed in AHDB project PC 229 (Mumford 2006) in combination with RT-PCR, which allows for the quick, low cost detection of PepMV, PSTVd and ToMV, whilst preserving the germination rate of the tested seed.

***Tomato spotted wilt virus* (*TSWV*)**

Hyperspectral imaging and deep learning

Hyperspectral imaging can be used to detect disease, nutritional and physiological issues before visible symptoms develop, allowing infected crops to be addressed quickly, limiting spread. Thermal and stereo visible light imaging has been demonstrated to provide an early rapid and non-destructive method of identifying powdery mildew (Raza, Prince *et al*. 2015).

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A hyperspectral imaging and outlier removal auxiliary classifier generative adversarial nets have been developed which can detect early infections with TSWV, with a high degree of accuracy (Wang, Vinson *et al*. 2019). This model can potentially be transferred to other virus pathogens of tomato.

Technological innovation and improvements in deep neural network have driven improvements in image recognition. A robust deep-learning based detector for the real-time identification of in-crop tomato issues has been developed to successfully identify nine pest and disease issues (Fuentes, Yoon *et al*. 2017). Although not currently able to detect viral issues, future work will expand the pest and disease profile detected by this technology. Imaging, combined with deep learning will likely represent the future of pest and diseased management in tomato crops.

Chemical and cultural management options

Chemical control

There are currently no chemical control options for any known virus, with all efforts focussed on controlling vectors and other routes of transmission. Chemical control is available to manage whiteflies, however insecticide resistance is widespread, and no chemical treatments are available which effectively control WFT in tomato. Due to maximum residue level (MRL) concerns, harvest intervals and the impact on natural predator populations, most pest control in tomato is biological in nature. Biocontrols are the most effective way to control viral vectors, and other pests (van Lenteren and Woets 1988), but need to be applied early, and in sufficient quantities before large pest populations become established.

Tomato spotted wilt virus (TSWV)

Thrips do not commonly occur in UK tomato production, but can occur on sites growing a mixture of plant types alongside tomato, including cucumber and ornamentals. However, thrips can become an issue in overwintered lit tomato crops. Thrips presence poses a difficult target for traditional chemical insecticide treatments, with adults and larvae often present in inaccessible locations. WFT have become resistant to the majority of chemical pesticides, including spinosad (Bielza, Quinto *et al*. 2007). WFT populations with resistance to all currently available chemical pesticides now exist.

Biocontrol options can provide some thrip control. Natural predators *Neoseiulus cucumeris* and *Amblyseius swirskii* are available for use in several plant types (Zilahi-Balogh, Shipp *et al*. 2007), and these are effective on both young thrips and larva stages. Plant extracts are also available, including azadirachtin, which is effective against WFT at the nymph stage. The effectiveness of biological control agents on different pest life stages means that their use

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needs to be planned and managed carefully, but currently most of these products are not authorised for use on commercial tomato in the UK.

Tomato spotted wilt virus (TSWV)

In outdoor grown crops in the USA, a later planting date has been demonstrated to provide improved WFT control (G. Riley and Pappu 2000), alongside the use of black (ultraviolet reflective) mulches which repel thrips (Tyler-Julian, E. Funderburk *et al*. 2012). Although effective, these practices would not be practical in UK indoor tomato crops.

Tomato chlorosis virus (ToCV) and Tomato infectious chlorosis virus (TICV)

ToCV and TICV are spread exclusively by species of whitefly. No varietal resistance to these viruses is available and whitefly control is the only option, however resistance to all groups of chemical insecticides is reported in *B. tabaci* (biotype B). *Encarsia formosa, Verticillium lecanii* (Mycotal®) and *Macrolophus pygmaeus* are all biocontrol agents currently authorised for use in the UK. These products are not completely effective and need to be used in a program including insecticides such as fatty acids, pymetrozine and spiromesifen.

Cultural control

Pre-cropping

Preventing the initial introduction of pathogens is the most important disease control strategy available. Once a plant is infected there is no cure, and as soon as visible symptoms have developed, it is possible that large areas of the crop are already infected. At this stage complete control is unlikely to be achieved.

All efforts are made by propagators and main-crop growers to prevent entry of tomato viruses and vectors onto commercial sites. Seed and suspect plant tissue samples can be tested at certified diagnostic laboratories before dispatch. At commercial sites, before plant arrival, the entire site architecture, including all equipment, will have been washed and then treated with appropriate disinfectants (at manufacturers recommended rates). Any disease ‘hot spots’ from the previous season will be dealt with and site hygiene protocols will be reviewed and updated. Some growers at risk of contracting PepMV may treat plants with a mild strain PepMV isolate at this time. In future mild-strains to other tomato viruses, including ToBRFV, may be developed to aid virus management.

During cropping

An integrated pest management (IPM) approach is the most effective way of managing viral outbreaks. Crops are monitored during the entire season including using sticky traps and

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lures for pest identification and quantification. Yellow and blue traps are available, with blue traps the most effective at attracting WFT (especially at low pest levels). Yellow traps catch other pest species, including whitefly. These traps are useful following plant arrival where thrips numbers may be too low to be reliably seen on plants (O'Neill and Bennison 2010). Thrip lures are also available, using pheromone attractants to lure thrips of several species (Teulon, Davidson *et al*. 2009). Alone these will not confirm the presence or absence of viruses, but will provide an indication of potential risk, based on pest numbers, if virulferous vectors are present. Samples of thrips/whiteflies can be sent away for analysis to determine if they contain viruses.

Mitigation against abiotic stress, optimising plant nutrition/health, and the promotion of beneficial microbial communities all contribute to viral resistance. Alone these are unlikely to reduce the likelihood of infection occurring, but these actions will help delay/overcome some viral effects.

Hand washing using soap and water is the primary control against disease transmission on commercial sites, alongside disinfectants, located at site entrances, including hand steriliser, foot dips and gloves. The efficacy of some hand sanitisers/disinfectants against certain pathogens is unclear and proven effective products must be used at all times e.g. Menno Florades for ToBRFV. Staff and equipment movement between houses/sites is minimised, with all equipment cleaned before the introduction to new areas. Shoe coverings, oversuits and gloves are made available to visitors who sign and follow the individual sites hygiene protocols. Visitors deemed to be high risk are restricted in their movement on, or are not permitted to visit sites. During periods of high risk, e.g. immediately following inoculation of the PepMV mild strain, or at times of newly emerging viruses e.g. ToBRFV, movement and access is limited further.

Where small numbers of plants are symptomatic of a virus, suspect plants are immediately bagged within the affected compartment, removed and placed in a covered skip, or disposed of away from site. This can include incineration, deep burial, or composting to a PAS100 standard. All affected plant material is removed, including the slab and propagation cube, as viruses including TSWV are systemic. Where practical and rapid diagnostic tests are available (LFDs), samples can be tested on site, or alternatively sent away to a diagnostic laboratory for confirmation (ELISA, RT-PCR). Affected areas are closely monitored until viral infection presence/absence is confirmed and a management plan considered.

Ongoing control

Sites at risk of infection from insect-vectored pests should treat any known viral reservoir species which may occur on, or around, the site, as these may act as potential inoculum

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sources. Tomato crops should not be grown in the same compartments as ornamental plants, however the use of indicator plants, e.g. petunia, may be grown for diagnostic purposes.

Close links exist between UK grower sites, the industry in Holland and the greater EU. In the UK, the British Tomato Growers' Association, represents the industry and two smaller grower groups, the Tomato Study Group (TSG) and Tomato Working Party (TWP) exist to share ideas, experiences and information regarding all components of tomato growing, including virus management.

Breeding for vector resistance and host resistance and/or tolerance

Variety choice is an essential consideration for virus management. Plant breeding has been successful in producing tomato varieties with high (HR) or intermediate (IR) resistance to common viruses, including ToMV, TMV and TSWV (Stevens, Scott *et al*. 1991, Vidavsky and Czosnek 1998); (Snodgrass and Ozores-Hampton 2014). The use of these varieties limits viral growth and development, under normal disease pressure. However, some plants may still exhibit minor symptoms under high disease pressure (Turini 2018). Varietal resistance is especially useful during seasons following severe viral infections, protecting crops, whilst allowing any potential residual viral load on-site to further diminish. It should be noted that variety choice is often a result of consumer demand, and supermarket requirements, rather than directly down to individual grower choice.

Pepino mosaic virus (PepMV)

Several strains of PepMV exist, with the CH2 and EU strains predominant in Europe. These strains demonstrate high homogeneity with the original Peruvian strain, the LP strain (Pagán, Cordoba *et al*. 2006). US1 and US2 strains have also been identified, with around 80% similarity to the LP strain (Maroon-Lango, Guaragna *et al*. 2005).

The US1 strain was identified in the UK in 2016, on commercial sites (Mayne and O'Neill 2016), and was more recently identified in Tenerife (Gómez-Aix, Alcaide *et al*. 2019). Multiple strains can, and often will, infect the same commercial site, and there is risk that the presence of additional strains is linked to greater fruit symptom expression (Mayne and O'Neill 2016). The presence of the US1 strain is currently low in the UK, but an increase in the presence of this strain, and the potential introduction of the US2 strain could put the industry at greater risk.

No varietal resistance to PepMV exists, but is under development (Soler, Lopez *et al*. 2011). Mild strains of PepMV have been developed which provide cross-protection (O'Neill 2014) through alignment-guided mutagenesis of the viral capsid protein (De Nayer, Goen *et al*. 2011, Chewachong, Miller *et al*. 2015). A vaccination strategy using a mild, stable Chilean

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PepMV isolate PMV®-01 is currently distributed by the Belgium company DCM (www.dcm-info.com). This isolate rapidly colonises inoculated plants providing cross-protection against more virulent PepMV strains, and is utilised by several UK growers who suffer from repeat PepMV infections. There is potential risk for recombination when using mild strain virus inoculums where other strains are present on site.

***Tomato spotted wilt virus* (*TSWV*)**

Resistance to TSWV is based on the presence of the SW5 resistance gene (Saidi and demudhar Warade 2008). Despite incorporation of the SW5 gene into several commercial varieties, <3% TSWV expression can still occur in resistant varieties under high disease loads (Turini 2018). In 2012 TSWV symptoms developed on several high resistance varieties in Italy and over half of plants became infected with severe symptoms developing (Crescenzi, Fanigliulo *et al*. 2015). This was confirmed to be a newly evolved TSWV SW5-resistence breaking strain. In 2018, a SW5 resistance breaking strain was identified in California, USA. Not yet reported in the UK, the introduction of resistance breaking strains poses an increased risk to the industry and resistance breeding will be necessary to overcome this.

***Tomato mosaic virus* (*ToMV*) *and Tobacco mosaic virus* (*TMV*)**

Highly resistant varieties exist for ToMV and TMV, containing the Tm-2 and Tm-2^2 genes which have been used to successfully control ToMV and TMV. Where resistance is not available seed and high health plant material is sourced from sources confirmed to be clear of infection.

***Tomato chlorosis virus* (*ToCV*) *and Tomato infectious chlorosis virus* (*TICV*)**

There is no resistance to ToCV or TICV in current commercial tomato varieties. Garcia-Cano *et al*, 2010 identified two sources of resistance to ToCV in a screen of wild tomato species. Resistance impaired both virus accumulation and symptom expression, even after artificial inoculation. Another screen tested 56 genotypes, with only one linage, IAC-CN-RT demonstrating high resistance via inoculation with ToCV virulferous *B. tabaci* (Mansilla, Bampi *et al*. 2017). Although not present in commercial varieties, this demonstrates the existence of resistance genes which could one day be incorporated.

***Tomato brown rugose fruit virus* (*ToBRFV*)**

No varietal resistance exists, with ToBRFV able to infect plants containing the Tm-2^2 resistance genes (Luria, Smith *et al*. 2017). There will be considerable focus by seed houses to develop resistant varieties to ToBRFV.

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***Southern tomato virus* (*STV*)**

It is currently unclear if STV infection alone is sufficient to lead to symptom development. Further research on this and mixed infections is necessary to identify if host resistance/tolerance exists.

Genetic modification and CRISPR technology

Although not legal in the UK, genetic modification has the capacity to develop partial, or full, virus resistance. (Kunik, Salomon *et al*. 1994) developed transgenic plants expressing the *Tomato Yellow Leaf Curl virus* (TYLCV) capsid protein (V1). All F1 plants subsequently inoculated with TYLCV (expressing the V1 gene) recovered following viral challenge. A similar technique transforming tomato plants using *Agrobacterium tumefaciens* increased the tolerance of tomato plants to *tomato leaf curl virus* (TLCV) compared to untransformed control plants (Raj, Singh *et al*. 2004). Tomato plants transformed with a binary vector containing a modified coat protein of the *Tomato mottle virus* (ToMoV) have also been developed leading to resistance (Sinisterra, Polston *et al*. 1999).

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Virus management in sugar beet

Sugar beet (*Beta vulgaris L. ssp. vulgaris*) is a crop with great economic importance, particularly in temperate areas of the world, where it is a valuable sucrose source. While the most common use for cultivated sugar beets is for refined sugar, there are several other uses. By-products from processing beets into sugar (pulp and molasses) are used as fibre rich supplemental feed for livestock. They can also be used in alcohol production, commercial baking and in pharmaceuticals. Treated waste water from processing can be used for crop irrigation and bi-products from beet processing plants are used to make lime for treating soil. Sugar beet surpluses have also been used to produce biobutanol in the United Kingdom.

The EU is the world's leading beet sugar producer, producing approximately 50% of global sugar beet volume, and contributing around a fifth of the global sugar production. Almost 18 million tonnes of beet sugar are produced in the Union each year, with approximately 3.3 million ha of sugar beet grown in 2017 (FAOSTAT). The majority of the crop is grown in the northern half of Europe, where the temperate climate is more suitable. Sugar beet has major advantages over sugar cane production; approx. 1/3 of the water use, saline tolerance and a short growing season, allowing the crop to rapidly adapt to changing climatic conditions, the volatile sugar commodity market and fluctuations in sugar prices. In contrast Sugar Cane takes around two years before sowings are productive and is therefore slower to react to fluctuations in the sugar market. As a consequence, it is likely that the demand for sugar beet globally will increase, however cropping and rotations will fluctuate in the future potentially influencing the evolution of pests, weeds and diseases – in particular viruses.

The sugar beet industry plays a critical part in the European rural and agricultural economy, however, the removal of EU sugar quotas in 2017 have contributed to a global sugar surplus and low market prices. In addition, a depressed world market, increased incidence of diseases and losses of key pesticides, have placed significant pressures on the EU market and its global competitiveness. In 2018, the European Commission enforced a ban on the three main neonicotinoids (clothianidin, imadacloprid and thiamethoxam) for all outdoor uses, due to mounting evidence that they are highly damaging to pollinators such as bees (Budge *et al.,* 2015; Gibbons *et al*., 2015; Godfrey *et al*., 2014; Goulson, 2013; Kessler *et al*., 2015; Nuyttens *et al*., 2013). The 2019 season will see the first crop grown without neonicotinoid seed treatments since the early 1990's, leaving the industry extremely vulnerable to the return of highly damaging viruses, such as the *Virus Yellows complex*, carried by *Myzus persicae* and other aphid vectors. The following section of the review will focus on key viruses that the sugar beet sector face in the UK, the consequences of limited chemistry to control vectors and the new and emerging technology that will help relieve future pressures on the industry.

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Current viruses present in the UK

More than 20 viruses are known to infect sugarbeet around the world from several different genera, including the Poleroviruses, Closteroviruses and the Benyviruses. Within this review we will be focusing primarily on the key current sugar beet viruses that are economically important to the UK, such as *Beet necrotic yellow vein virus* (BNYVV), otherwise known as *Rhizomania* and the Virus yellows complex which is made up of *Beet yellows virus* (BYV), *Beet mild yellowing virus* (BMYV) and *Beet chlorosis virus* (BChV). Changes in the use of chemistry is likely to change their relative importance and of previously minor viruses. However, a more comprehensive list of viruses which can affect the UK crop can be found in Table 38.

Table 38. Summary of past and present Sugar beet viruses in the UK.

Virus	Acronym	Genus	Mode of Transmission
Beet necrotic yellow vein virus (Rhizomania)	BNYVV	*Benyvirus*	*Polymyxa betae, Rubbing of leaves*
Beet soil-borne virus	BSBV	*Benyvirus*	*Polymyxa betae*
Beet virus Q	BVQ	*Pomovirus*	*Polymyxa betae*
Beet soil-borne mosaic virus	BSBMV	*Benyvirus*	*Polymyxa betae*
Beet mosaic virus	BMV	*Potyvirus*	*Aphid transmitted*
Beet yellows virus	BYV	*Closterovirus*	*Aphid transmitted*
Beet mild yellowing virus	BMYV	*Polerovirus*	*Aphid transmitted*
Beet chlorosis virus	BChV	*Polerovirus*	*Aphid transmitted*
Beet curly top virus	BCTV	*Crutovirus*	*Seed transmitted*
Beet cryptic virus	BCV	*Alphacryptovirus*	*Aphid transmitted*
Beet leaf curl virus	BLCV	*Nucleorhabdovirus*	*Aphid transmitted*
Beet yellow net	BYN	*Luteovirus*	*Mechanical and aphid transmission*

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Beet yellow stunt	BYSV	*Closterovirus*	*Aphid transmitted*
Cucumber mosaic virus	CMV	*Cucumovirus*	*Mechanical and aphid transmission*

Virus Yellows

Virus yellows are caused by a complex of viruses causing severe yellowing to the crop canopy and yield losses, which include *Beet mild yellowing virus* (BMYV), *Beet yellows virus* (BYV) and *Beet chlorosis virus* (BChV) (Stevens *et al*., 2006). Virus yellows is historically one of the most important diseases of sugar beet, due to its severe impact on sugar yield and processing quality. Between 1980-1995 virus yellows was estimated to decrease sugar yield in the UK by approximately 2% annually, costing the industry over £10 million in the absence of control measures and £5.5 million per year when treatments were applied (Jaggard *et al.,* 1998).

Beet yellows virus

Beet yellows virus (BYV, family: *Closteroviridae*, genus: *Closterovirus*), is a member of the virus yellows complex which infects sugar beet. The virus is primarily found in the Mediterranean, but can also be found in the UK and is transmitted in a semi-persistent manner predominantly by the green peach aphid (*Myzus persicae*). Infection with BYV significantly reduces total plant weight by 20%, which is primarily due to a 25% reduction in storage root growth. Infection does not reduce extraction depth in field experiments, despite decreasing lateral root growth in the glasshouse (Clover *et al*., 1999). The growth reduction in infected plants results from a decrease in net photosynthesis due to damaged photosynthetic mechanisms and an increase in the proportion of light intercepted by yellow leaves (Clover *et al*., 1999). Russel (1963) concluded that BYV was able to reduce sugar yield by approximately 3% every week it was infected. Smith & Hallsworth (1990) showed that early infections in the season can cause up to 47% yield losses in comparison to late infections where reductions of 30% were more typical. The extent of yield reduction is determined by the cultivar,the virus strain and the harvesting schedule, which may form part of future management approaches. Infection is also linked to increases in root impurities such as sodium, potassium and amino-nitrogen (Smith and Hallsworth, 1990), which significantly represses sugar extraction efficiency.

Beet mild yellowing virus

Beet mild yellowing virus (BMYV, family: *Luteoviridae*, genus: *Polerovirus*), is usually the predominant cause of yellowing in sugar beet in England and can decrease sugar beet yield by up to 30% (Smith & Hallsworth, 1990). The virus is persistently transmitted by the aphid

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Myzus persicae and forms part of the virus yellows complex, alongside BYV and BChV. Yield losses associated with late season infection are significantly less at 4-15% (Smith *et al*., 2004). BMYV is generally distributed in the northern and western regions of Europe (Stevens *et al.,* 2005) and due to its relatively wide host range, BMYV is able to overwinter in *Beta* species and common arable weeds such as *C. bursa-pastoris, Stellaria media, Senecio vulgaris, Spergula arvensis* and *Veronica* spp. (Jadot, 1974; Stevens *et al.,* 1994; Patron, 1999). This phenomenon significantly increases the risk of large yield losses due to a widely distributed early infection when high levels of *Myzus* are present. As well as significantly decreasing root weights and sugar yields, BMYV also increases juice impurities, such as sodium which can have a detrimental effect on the factory processing of the beet (Smith *et al*., 2004) and therefore the economic sugar extraction by UK growers.

Beet chlorosis virus

Beet chlorosis virus (BChV, family: *Luteoviridae*, genus: *Polerovirus*), which is persistently transmitted by the aphid *Myzus persicae*, is part of the virus yellows complex in sugar beet and causes interveinal yellowing as well as significant yield loss. BChV was first described as a second strain of BMYV by Stevens in 1994 in the UK and by Liu in the USA (Stevens, *et al*., 1994, Duffus & Liu, 1991). The International Committee for the Taxonomy of Viruses (ICTV) went on to assign the virus as a distinct member of the genus Polerovirus in 2002, based on its molecular, serological and biological traits (Stevens *et al*., 2004). Field studies have shown that BMYV is more damaging than BChV to root and sugar yields early in the growing season (May & June), but late BChV infections (July) has a greater impact on yield (Stevens *et al*., 2004). As in the case of BMYV, BChV can also increase juice impurities, which can detrimentally effect factory processing (Stevens *et al.,* 2004). In 2005, Stevens *et al*., published results from a large scale screen of BChV isolates in 10 countries over three continents. BChV, originally identified in the UK and USA was identified in France, Spain, the Netherlands, the USA and Chile during the study. In the UK over 25% of the UK polerovirus isolates were identified as BChV in 2002, an increase of 14% when compared to previous screens in 1990 (unpublished data).

Beet mosaic virus

Beet mosaic virus (BtMV, family: *Potyviridae*, genus: *Potyvirus*) is a non-persistently aphid-transmitted potyvirus infecting mainly sugar beet and its close relatives. BtMV is one of the most widely distributed sugar beet viruses and is likely to be present in all major beet-producing regions of the world. Beet mosaic virus is not normally considered economically important as yield losses associated with the disease are generally low, however, if plants are infected at an early growth stage yield losses can be up to 10% (Bennett, 1964).

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Beet necrotic yellow vein virus

Beet necrotic yellow vein virus (BNYVV, family: *Benyviridae*, genus: *Benyvirus*), is a multipartite single-stranded positive-sense RNA virus, more commonly known as Rhizomania (see McGrann *et al*., 2009 for a review). It is a soil-borne disease and was first discovered in the UK in 1987 (Asher, 1987). The disease has since spread throughout the UK beet growing area. The virus is transmitted by viruliferous zoospores of the soil-borne plasmodiophorid protozoa, *Polymyxa betae* (Tamada & Baba, 1973). Rhizomania ('root madness or beardness') is one of the most devastating diseases of sugar beet, lowering root yield by up to 90% in susceptible beets depending on the virulence of the BNYVV type (Johansson, 1985). The disease is characterized by the extensive proliferation of lateral rootlets along the main taproot, necrosis of the vascular bundle and severe stunting of the plant.

Since 1994, three major types of BNYVV have been described and are referred to as A-type, B-type and P-type. The three main variants are not serologically distinct and can only be differentiated by variation found within RNA1, RNA2, RNA3 and RNA4, as well as by phylogenetic relationships (Koenig *et al.,* 1995; Kruse *et al.,* 1994; Schirmer *et al.,* 2005). An additional J-type was proposed for Asian strains which contain an additional RNA5 (Shirmer *et al.,* 2005). The A-type and B-type are the most common strains, with the A-type prevalent world-wide and the B-type found primarily in France, Germany, Japan, UK and Sweden (Koening *et al.,* 1995; Lennefors *et al.,* 2000; Miyanishi *et al.,* 1999). A and B-type strains of BNYVV are highly conserved, with nucleotide identity in the range of 96-99%, however there are regions, such as RNA2 which have sufficient sequence diversity to develop PCR-based diagnostics (Ratti *et al.,* 2005; Kruse *et al*., 1994; Mitanishi *et al*., 1999). The RNA3-encoded P25 is responsible for symptom development and virus aggressiveness (Koenig *et al*., 1991; Tamada *et al*., 1999) and also exhibits high sequence diversity within a 4 amino acid stretch between positions 67-70, termed the hypervariable tetrad. The P-type was first isolated in the Pithiviers area in France and was found to also contain the RNA5 and an additional pathogenicity factor P26 (Koenig *et al*., 1995). The ability of this strain to overcome the Rz1-mediated resistance was demonstrated by Pferdmenges *et al*., 2008.

Resistance breaking (RB) strains of BNYVV have been identified in a number of countries, including the UK. The virulent AYPR strain of Rhizomania has been identified in a total of 31 fields in the UK between 2007 – 2012, with a significant cluster around Woodbridge in Suffolk and cases in Norfolk and Essex. There is anecdotal evidence to suggest that such isolates originated from potato washings from boats in the 1950's. The holds of ships - which originated from potato growing areas in Holland known to be infected with AYPR - were washed out and the washings spread on local land. The AYPR strain can reduce root yields

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by up to 70% and sugar percentages by 10-22% (Stevens and Mothersole, 2011) in susceptible varieties. Varietal susceptibility to AYPR was observed and varieties which were identified as more resistant were found to carry an additional resistance gene, termed Rz2. All Sugar beet varieties on the Recommended List carry Rz1 resistance and there are now varieties available which Rz1/Rz2 resistance varieties are specifically recommended for growing where AYPR has been confirmed in both the sugar beet and fodder beet sector, however there are still instances where farmers continue to grow susceptible varieties of Fodder beet in high risk areas. Further advances in breeding for BNYVV resistance in sugar beet will be discussed in section the section on 'Breeding for aphid resistance and host resistance and/or tolerance'.

New emerging viruses

Minimal work is currently being carried out to monitor sugar beet viruses in the UK, within the crop, the key vectors and alternative hosts. It is therefore essential that more effort is made in the future to monitor pressure from viruses in sugar beet, particularly those which are carried by aphid vectors, inorder for the industry to keep on top of potential threats.

Vectors of key viruses

Sugar beet viruses are transmitted by a number of vectors. Whilst the majority of virus infections in sugar beet globally are transmitted by aphids (e.g. *Beet yellows virus*, *Beet mild yellowing virus, Beet chlorosis virus*), there are other viruses which are transmitted by soil borne vectors (e.g. *Beet necrotic yellow vein virus*) or can be transmitted mechanically (e.g. *Beet yellow net*). Aphid transmitted sugar beet viruses may be transmitted in a persistent (e.g. *Beet chlorosis virus, Beet mild yellowing*) or a non-persistent manner (e.g. *Beet yellows virus*). Persistently transmitted sugar beet viruses infect the vector aphid for its lifetime and any plants on which such an aphid then feeds will be at risk of acquiring the virus. Non-persistently transmitted sugar beet viruses can only be transmitted immediately after aphids have fed on an infected plant. Vectors of the key sugar beet viruses predominantly found in the UK can be found in Table 39.

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Table 39. Vectors of the key UK sugar beet viruses

Virus	Vector
Beet yellows virus (BYV)	Aphid transmission e.g. *M. persicae, A. Fabae.*
Beet mild yellowing virus (BMYV)	Aphid transmission *e.g. M. persicae, A. fabae, M. euphorbiae.*
Beet chlorosis virus (BChV)	Aphid transmission e.g. *Myzus persicae*
Beet mosaic virus (BtMV)	Mechanical and Aphid transmission e.g. *M. persicae, A. pisum, A. fabae, M. euphorbiae, M. dirhodum, R. padi*.
Beet necrotic yellow vein virus (BNYVV)	*Polymyxa betae* transmission, rubbing of leaves

Beet yellows virus

Beet yellows virus is transmitted semi-persistently by more than 22 species of aphid (Watson, 1946; Sylvester, 1956; Kennedy *et al*., 1962), but *Myzus persicae* and *Aphis fabae* are the key vectors in a natural field environment. Adult aphids are the most effective at transmitting the virus, but all instars are capable. Apterous *M. persicae* is twice as efficient at transmitting at transmitting BYV as *A.fabae* (60% and 34% respectively) and apterous forms of *A. fabae* were equally efficient in transmitting BYV as compared with alate *A. fabae* (Limburg *et al*., 1997). The virus can be retained by the vector for up to three days, with a half-life of about 8 hours. Acquisition feeds of more than 12 hours and test feeds of at least 6 hrs are necessary for optimal transmission. BYV is not transmitted to progeny of vectors or retained after moulting and is non-transmissible through seed and pollen (Watson, 1960).

Beet mild yellowing virus

Beet mild yellowing virus is transmitted predominantly from plant to plant by green peach aphids (*Myzus persicae*) at a transmission rate of 28.6% (Russel, 1965; Schliephake *et al.,* 2000). Like other poleroviruses, BMYV is transmitted in a persistent manner and is limited to the vascular tissue of its hosts and mechanical inoculation is only possible in mixed infections with umbraviruses (Mayo *et al.,* 2000). Schliephake *et al.,* (2000) carried out further transmission tests, testing 24 different aphid species for the ability to transmit BMYV in sugar beet. In addition to *Myzus persicae*, both *Aphis fabae* and *Macrosiphum euphorbiae* were both found to transmit BMYV at 1.1% and 1.8% transmission efficiency respectively.

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Beet chlorosis virus

Beet chlorosis virus (BChV) is a persistent virus transmitted by the green peach aphid, *Myzus persicae.* Little information is available on the transmission of this virus or the range of aphids which are able to transmit the virus. Unlike its close relative, BMYV, *Macroziphum euphorbiae* is not able to transmit both British and American BChV isolates (Stevens *et al*., 2005). A systematic study of beet polerovirus vector specificity, particularly BChV, would be of great use, particularly in light of the loss of neonicotinoid seed treatments to control vectors.

Beet mosaic virus

Beet mosaic virus (BtMV) is a non-persistent aphid-transmitted potyvirus. More than twenty-eight species of aphid are able to transmit the virus (Kennedy *et al*., 1962), but the principle vectors in the field are *Myzus persicae* and *Aphis fabae* (Russell, 1971; Dusi & Peters, 1999). Acquisition and inoculation thresholds are reached within seconds of feeding, with no latent period and viruliferous *Myzus persicae* can retain the virus for at least 16 hours (Dusi, 1999). Due to short acquisition and transmission periods, the spread of the virus has been shown to occur mainly around foci (Shukla *et al*., 1994), resulting in steep gradients. Secondary spread usually occurs over short distances. The virus is also easily transmitted by mechanical inoculation (Cockbain *et al*., 1963), but is not seed transmitted.

Beet necrotic yellow vein virus

Polymyxa betae, is the soil borne vector for *Beet necrotic yellow vein virus*. *P. betae* is an obligate parasite which forms two types of spores during the lifecycle: motile zoospores and thick-walled resting spores, cytosori (Keskin *et al*., 1964). The zoospores attach to rootlets during infection where a tubular structure is formed and penetrates the host cell and the contents of the zoospore is then injected into the plant cell (Keskin & Fuchs, 1969). At this stage, BNYVV can be transferred to the plant cell if the zoospore is carrying the virus. *P. betae* can move into the multiplication phase where sporangia are formed and new zoospores are produced. The zoospores can actively swim to new root cells and infect them when soil moisture is high. The infection cycle can be completed within 60 hours under optimal conditions (soil pH between 6 & 8, high water content and a temperature of +25ºC). *P. betae* can persist in the soil for 20-25 years due to the formation of resting spores which can go onto germinate under favourable conditions and release zoospores. In general, *P. betae* causes limited damage to sugar beet plants, but the viruses that it can carry and transmit, such as BNYVV, BSBV, BSBMV can be highly damaging. In addition, experiments carried out in the late 1980's and early 1990's showed that *P. betae* isolates can show significant differences in virulence and several virulent isolates were shown to reduce the growth of roots in the sugar beet plant in particular (Gerik & Duffus, 1988; Blunt *et al*., 1991; Kastirr *et al*.,

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1994). Fan *et al*., (2015) reported that the leaves of *Beta macrocarpa* could be inoculated with BNYVV infectious clones without requiring *P. betae* as a vector. This novel method avoids the variation associated with using *P. Betae* as a vector.

Alternate hosts of key viruses

Several weed and ornamental species act as natural reservoirs for sugarbeet viruses, providing a valuable habitat for over wintering of vectors such as *Myzus persicae* and an alternative host for virulferous vectors to spread viruses to the following crop. Therefore, the loss of pesticide and herbicide products due to resistance and/or changes to regulation poses a major challenge to the sugar beet industry, with regards to the future control of viruses. It is therefore critical that weed control is given equal importance to vector control, when future integrated management control strategies are formed.

Beet yellows virus

Beet yellows virus is capable of infecting 121 species of 15 plant families, but the most infectible species are in the *Chenopodiaceae, Amaranthaceae, Aizoceae* and *Caryophyllaceae* families (Duffus, 1973). Aside from *Beta vulgaris, Tetragonia expansa* is considered an efficient diagnostic and propagation species, exhibiting clearing and yellowing of veins of younger leaves, leaf yellowing and distortion of older leaves. Plants also become dwarfed. *Montia perfoliata* can be aphid inoculated and symptoms are comprised of red-rimmed necrotic spots and chlorosis in older leaves. Mechanically inoculated leaves exhibit local necrotic lesions, but no systemic infection (Russel, 1970). *Chenopodium amaranticolor* and *C. foliosum* exhibit chlorotic local lesions and acute stunting, distortion, vein clearing and premature death respectively (Russel, 1970). BYV infection of *Nicotiana clevelandii* is symptomless, but systemic.

Beet mild yellowing virus

Host-range of BMYV was described by Russel (1965a) and by Björling and Nillson (1966). Russel (1963,1965a) recommended for the differentiation of BYV and BMYV plant species *Capsella bursa-pastoris* (Medik), which is sensitive to BMYV but not to BYV, *Chenopodium foliosum* (Moench) ASCH, which is sensitive to BYV and not BMYV and *Claytonia perfoliata* (Donn) which is sensitive to both viruses. In addition, *C. foliosum* (Moench) ASCH and *Tetragonia expansa* (Murr) are also sensitive to BMYV. Additional hosts for the virus can be seen in Table 40.

Beet chlorosis virus

Beet chlorosis virus displays a significantly narrower host range than BMYV and is generally restricted to *Beta* species. However, *Chenopodium capitatum, Spergula arvensis* and

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Spinacia oleracea can also act as hosts. Unlike BMYV, BChV cannot infect *Capsella bursa-pastoris*, *Stellaria media, Senecio media* and *Montia perfoliata*, so these species can be used to differentiate between the two closely related viruses (Hauser *et al*., 2002).

Beet mosaic virus

Host range of BtMV is fairly wide and it infects mainly within *Chenopodiaceae, Solanaceae* and *Leguminosae.* Several species within ten dicotyledonous families have been infected experimentally (Bennett, 1949). Diagnostic species include *Beta vulgaris* showing vein clearing in young leaves and light/dark green mottling in older leaves and *Spinacea oleracea*, where small yellow flecks, joining to form large chlorotic lesions, appear on the youngest leaves. Older leaves become progressively more chlorotic and necrotic. Sugar beet is considered the best propagation species for maintaining cultures.

Beet necrotic yellow vein virus

BNYVV and its vector *P. betae* have limited host ranges, with just a small number of members from the *Amaranthaceae, Asteraceae, Caryophyllaceae, Chenopodiaceae, Portulacaceae, Solanaceae* and *Poaceae* recorded as alternative hosts for the virus (Tamada and Baba, 1973; Barr and Asher, 1992; Hugo *et al.,* 1996; Legreve *et al.,* 2005; Mouhanna *et al.,* 2008). Host studies on *Brassicaceae* have also been carried out but, to date, only *Raphanus raphanistrum* has been confirmed as a host (Kutluk Yilmaz *et al.,* 2016).

Table 40. Alternate host species for key sugar beet viruses in the UK.

Virus	**Alternate/diagnostic host**
Beet yellows virus (BYV)	*Chenopodiaceae, Amaranthaceae, Aizoaceae, Caryophyllaceae, Tetragonia spp.Nicotiana clevelandii,*
Beet mild yellowing virus (BMYV)	*Beta* species, *Brassica napus* var. napobrassica, *L. purpureum*, *Capsella bursa-pastoris, S. media, S. arvensis, S oleracea, S. media, S. vulgaris, Plantago spp, Viola arvensis, M. perfoliata.*
Beet chlorosis virus (BChV)	*Beta* species, *C. capitatum, S. arvensis* and *S. oleracea.*
Beet mosaic virus (BtMV)	*Chenopodiaceae, Solanaceae* and *Leguminosae*
Beet necrotic yellow vein virus (BNYVV)	*T. expansa, G. globose, B. macrocarpa, C. amaranticolor, N. bethamiana.*

Table 40 shows the most common alternate or diagnostic host species for the key viruses only. This list should not be considered exhaustive.

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Detection and Identification of Viruses

Visual symptoms between sugar beet viruses, particularly the viruses within the virus yellows complex are extremely similar. It is therefore crucial that techniques are available to detect and identify key viruses. Techniques currently available can be found in Table 4.

Table 41. Methods for detection for key sugar beet viruses

Relevant virus	Detection method
BYV	ELISA, Scanning transmission electron microscopy, Western blot, RT PCR, Imunocapture RT PCR, Northern blotting
BMYV	ELISA, Scanning transmission electron microscopy Immune-specific electron microscopy, Microprecipitin, RT PCR, Imunocapture RT PCR
BChV	ELISA, RT PCR, Imunocapture RT PCR
BtMV	ELISA, RT PCR
BNYVV	Scanning transmission electron microscopy , Laser scanning confocal microscopy of infectious virus clones tagged with GFP, Immune-specific electron microscopy Microprecipitin tests , Tissue print-immunoblotting, Lateral flow kit , Northern blotting , One-step RT PCR, Nested One-step PCR, Immunocapture RT-PCR , Taqman RTPCR , RFLP, Deep sequencing transcriptomics

Detection and Identification Methods Based on Biological Properties

Beet yellows virus

Depending on the cultivar and the virus strain, BYV symptoms may vary from mild to very severe. Following infection with strongly pathogenic strains, the first symptoms consist of vein clearing, progressing to vein chlorosis and vein yellowing (vein etching), often accompanied by necrosis. After the appearance of diffuse chlorotic spots, a general yellowing expands from the leaf tips. The leaf colour may vary from pale yellow to dirty brownish-yellow and the leaf texture is rigid and brittle (Virus yellows monograph, IIRB). The virus particles of BYV are flexuous and are 1,250nm long, with an outer diameter of 10-12nm and a hollow core of 2-4nm (Leyon, 1951; Brades and Zimmer, 1955; Horne *et al*., 1959). The virus is made up of a 15.5kb positive sense RNA genome and five proteins. Identification of BYV by ELISA (in plant tissue: Chevallier and Putz, 1982; Roseboom and Peters, 1984; in single aphids: Rogov *et al*., 1993), western blot (He & Ro, 1997), scanning transmission electron microscopy (Cronshaw *et al*., 1966; Chevallier *et al*., 1983), have been demonstrated.

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Beet mild yellowing virus

In contrast to BYV, BMYV does not cause vein clearing or vein etching. The first symptoms consist of diffuse chlorotic areas on fully-expanded leaves. The affected areas expand and eventually coalesce, starting from the leaf tip or edge. The discolouration changes from a faint chlorosis to a brilliant yellow-orange, contrasting with the lemon-yellow produced by BYV. The leaf texture is brittle and thickened. Damage caused by the virus can predispose the infected plant to secondary attack by *Alternaria* sp, which can produce large necrotic black spots which may destroy the leaf (Virus yellows monograph, IIRB). It is extremely difficult to purify BMYV for structural characterisation, however, Chevallier *et al*., (1983) demonstrated that BMYV particles have a diameter of 26 nm and a total mol. wt. of 6.5(_+ 0-45) x 106. The mol. wt. of the protein subunit is about 24000. The genome consists of a single strand of positive-sense RNA with a molecular weight of 2 x 10^6. It contains six open reading frames (ORF) of which the 3'-proximal ORFs encode for the coat protein, putative movement protein and the read through domain. The 5' end has three overlapping ORFs (Guilley *et al*., 1995). Identification of BMYV by ELISA (in plant tissue: Chevallier & Putz, 1982; in single aphids: Stevens *et al*., 1991; Stevens *et al*., 1995), scanning transmission electron microscopy (Chevallier *et al*., 1983), immune-specific electron microscopy and microprecipitin tests (Govier, 1985) have been demonstrated.

Beet chlorosis virus

BChV induces specific symptoms in the form of chlorotic and necrotic spots on the older leaves, together with vein yellowing. Infection can result in a sugar yield reduction of between 8-24% depending on the developmental stage of the crop at infection (Stevens *et al*., 2004). BChV is serologically similar to BMYV, with only 10% diversity in the amino acid sequences of the coat protein. However ORF 0 of BChV was found to have little homology with BMYV. Hauser *et al.,* (2001) carried out a molecular characterisation of the American isolate BChV-California and the European isolate BChV-2a. The publication reported a genetic organisation and expression typical of other Polerovirus members including 6 open reading frames (ORFs) and determined that BChV is a distinct species of the Polerovirus genus. Identification of BChV by ELISA has been demonstrated (Kozlowska-Makulska *et al*., 2007), with several companies selling commercial BChV ELISA kits.

Beet mosaic virus

BtMV causes mottling symptoms on beets, similar to other mosaic viruses seen on plant species. Initial symptoms are the appearance of chlorotic spots or rings on young leaves with green centres and vein clearing. Further growth will only show mottling symptoms. The mosaic pattern usually consists of irregular patches of various shades of green. As the plant

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matures the symptoms become less obvious. The virus is a member of the potyvirus group and causes the formation of intracellular cytoplasmic inclusions (pin wheels) which can be detected by light microscopy (Hoefert, 1969). The virus particles of BtMV are flexuous, filamentous rods about 695-770nm long and 13nm in diameter. The viral genome of BtMV comprises 9591 nucleotides, excluding the 3' terminal poly (A) sequence, and contains a single open reading frame (ORF), encoding a single polyprotein of 3086 amino acid residues. The deduced genome organization is typical for a member of the family Potyviridae and includes 10 proteins: P1, HC-Pro, P3, 6K1, CI, 6K2, NIa-VPg, NIa-Pro, NIb and coat protein (CP) (Nemchinov *et al*., 2004). Identification of BtMV can be detected by ELISA, with several companies selling commercial BtMV ELISA kits.

Beet necrotic yellow vein virus

The disease is characterized by the extensive proliferation of lateral rootlets along the main taproot, necrosis of the vascular bundle and severe stunting of the plant. BNYVV consists of rod-shaped particles (20nm in diameter) of four different size classes (390, 265, 100 and 85nm in length) (Putz, 1977; Putz *et al*., 1988). The virus is composed of four genomic messenger-like RNAs of 6.8, 4.7, 1.8 and 1.5kb with an additional fifth RNA found frequently in Asia (Tamada *et al*., 1989) varying from 1.34 – 1.35 kb. Identification of BNYVV by ELISA (Henry *et al*., 1992; Pferdmenges, 2007; Žižyte *et al*., 2009), scanning transmission electron microscopy, laser scanning confocal microscopy (infectious virus clones tagged with green fluorescent protein (GFP)) (Erhardt *et al*., 2001), western blot (Torrance *et al*., 1988; Žižyte *et al*., 2009), immune-specific electron microscopy, microprecipitin tests (Žižyte *et al*., 2009) and tissue print-immunoblotting (Kaufmann *et al*., 1992) have been demonstrated. There is also a commercialised lateral flow kit available which was produced by Central Science, Laboratory, York, GB. In addition, Mutasa-Gottgens *et al*., (2003) developed polyclonal antibodies specific to *Polymyxa betae*, the vector for BNYVV which can be used to identify the vector using ELISA. A comprehensive review of diagnostic methods for BNYVV was written by the European and Mediterranean Plant Protection Organisation (2006).

Detection and Identification Methods Based on Viral Nucleic Acid

*Beet yellows virus (BYV)*Detection of BYV has been demonstrated using RT PCR in aphids and plant tissue, via Northern blotting and immunocapture RT PCR (Stevens *et al*., 1997; Kundu, 2004; Peremyslov *et al*., 2004).

Beet mild yellowing virus (BMYV)

Detection of BMYV has been demonstrated using RT PCR (Hauser *et al.,* 2000b) and immunocapture RT PCR in aphids and plant tissue (Vinanó and Stevens, 2007). In 2013,

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Klein *et al*., developed a BMYV infectious clone which successfully infected a range of plant species including sugar beet. In addition, Stevens and Vigano (2007) developed an infectious BMYV clone which was tagged with Green Flourescent Protein (GFP).

Beet chlorosis virus

Detection of BChV has been demonstrated using RT PCR (Hauser *et al*., 2000b) and immunocapture RT PCR in aphids and plant tissue (Vinanó and Stevens, 2007).

Beet mosaic virus

Beet mosaic virus (BtMV) has not been thoroughly studied as it is not considered particularly damaging to sugar beet yield. However the detection of BtMV has been demonstrated using RT PCR (Nemchinov *et al*., 2004).

Beet necrotic yellow vein virus

Detection of BNYVV has been demonstrated using Northern blotting (Erhardt *et al*., 2001) one-step RT PCR, nested One-step PCR and immunocapture RT-PCR (Morris *et al*., 2001; Harju, 2003; Harju *et al*., 2005; Yardimic & Kiliç, 2011; Yilmaz 2019). Details of a Taqman RTPCR were provided in a Rhizomania diagnostics review written by the European and Mediterranean Plant Protection Organisation (2006). A multiplex PCR that can detect BNYVV, BSBV, *Beet virus Q* and *Polymyxa betae* has also been developed (Meunier *et al*., 2003). Yilmaz (2019) used Restriction fragment length polymorphism (RFLP) analysis to study BNYVV isolates in Turkey to determine which isolate types were more prevalent in different growing regions. Fan *et al*., (2014) demonstrated the use of deep sequencing transcriptomics to reveal insights into the responses of *Nicotiana benthamiana* to infection with *Beet necrotic yellow vein virus* infections.

Novel technologies

The potential for hyperspectral sensors and drone imaging techniques has grown significantly with regards to detecting and identifying plant disease in the field (Sankaran *et al.,* 2010; Mahlein *et al.,* 2012; Wahabzada *et al.,* 2015; Thomas *et al*., 2018). The majority of publications reporting such techniques in sugar beet has been focused on detecting and identifying fungal diseases, such as *Cercospora beticola*, rust and powdery mildew (Mahlein *et al.,* 2012). However, in 2003 hyperspectral leaf reflectance and multispectral canopy reflectance were used to study the physiological differences between healthy sugar beet and sugar beet infected with BNYVV. In the presence of declining nitrogen levels it was reported that BNYVV infected plants showed significantly lower chlorophyll and carotenoid levels, with the ratio of betacynanins to chlorophyll significantly increased in diseased sugar beet when

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estimated from canopy spectra (Steddom *et al.,* 2003). These results indicated that remote sensing technologies could be used to detect Rhizomania, however much work is still to be done to develop this technology. At present there is little to be found in the literature regarding the use of drone imaging to detect and quantify virus yellows symptoms in sugar beet. However, work is currently being carried out in the UK to develop a virus yellows phenotyping method capable of quantifying yellowing symptoms via drone images to aid genetic mapping studies (James *et al.,* unpublished data).

Modelling

Research to develop virus yellows forecasting models in sugar beet began in the 1960's, where it was discovered that a close relationship existed between temperature in winter and early spring and virus incidence in sugar beet crops at the end of August (Hurst, 1965; Watson, 1966). Weather and aphid number data in the field went on to be used to make predictions of virus yellows incidence in the following sugar beet crop (Watson *et al.,* 1975). Harrington *et al*., 1989 improved the forecast by including aphid flight data from the 12.2m-suction trap situated at Broom's Barn, allowing a preliminary forecast to be issued before crops are sown and enabling farmers to make early changes to control strategies if necessary. In 1998 this forecasting method was further revised by Werker *et al*., to include the relationship between the population dynamics of *Myzus persicae* and the spread of virus yellows. In 2004, Qi *et al*. also included the wide-spread use of neonicotinoid seed treatments into forecast equations. The recent loss of neonicotinoid seed treatments in sugar beet increases the importance of accurate aphid forecasting, as timing of foliar insecticide applications will be key in future seasons to ensure that good control of aphids and virus yellows is achieved. It will therefore be critical that forecasting tools in the UK and Europe are updated regularly in the future.

Chemical and cultural management options

Virus yellows

The risk of viruliferous *Myzus persicae* entering sugar beet crops in the UK and spreading virus yellows is present every season and so growers have been encouraged over the last thirty years to adopt an integrated control programme, which includes the use of insecticides, the removal of infection sources and sowing early. By sowing early there is a greater chance that plants will have matured before peak aphid migration, as the yellow-green colour of leaves on young beet plants attract *M. persicae* (Williams, 1995).

In 1991, the UK sugar beet industry saw the first generation of neonicotinoid seed treatments become available, providing excellent control of aphids for up to 10 weeks after sowing and

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protecting the crop from the threat of virus yellows during the early phases of crop growth (reviewed in Draycott *et al*., 2006). Use of the technology quickly became widespread throughout the industry (>90% of the crop treated with neonicotinoid based seed treatments; Stevens *et al*., 2012) and incidences of virus yellows fell to almost undetectable levels. In April 2018, a majority of EU Member States endorsed a Commission proposal to restrict the use of imidacloprid, clothianidin and thiamethoxam to use in greenhouses only, due to an increased risk to bees identified by the European Food Safety Authority (EFSA). The implementation of this legislation in 2019 has led to the first year since the 1990's where the sugar beet crop has been grown in the UK without the protection of a neonicotinoid based seed treatment. Teppeki (Flonicamid) is currently the only product fully registered for use on sugar beet in the UK to control aphids, with one spray permitted up to the 12-leaf stage. Once applied the crop will see protection for up to 21 days, however, aphid forecasting and timing of application is key as flying aphids could be a risk for up to 12 weeks. On the 18th April 2019 The British Beet Research Organisation (BBRO) secured emergency use of thiacloprid on the 2019 sugar beet crop in order to manage the risk of virus yellows, however this authorisation expires on the 15th August 2019. BBRO have advised farmers not to use pyrethroids or carbamates to control aphids in sugar beet crops, due to high levels of insecticide resistance to these actives and to prevent the chemistry from lowering populations of beneficial insects. It is not known what chemical control options will be available moving into 2020 and therefore it remains to be seen how severely the UK sector will be damaged due to the possible resurgence of virus yellows going forward.

During the 1970's significant effort was made to develop biological control agents for viral vectors such as *M. persicae*. For example techniques for rearing of aphid parasites (Adashkevich *et al*., 1975; Simpson *et al*., 1975), chryosopids (Morrison *et al*., 1975; Abashkin and Yazlovetskii, 1977) and coccinellids (Timofeeva, 1974) were developed with the aim to supply as a biological control programme in crops such as sugar beet. Trials in the US carried out to control aphids in potato crops showed limited control of *M. euphorbiae*, but achieved around 85% control of *M. persicae* (Shands *et al*., 1972). At the time it was considered not an economically feasible alternative to insecticides, as it was believed that predator larvae introduced during the early stages of the *M. persicae* cycle would starve before making a significant impact. Attempts to use pathogenic fungi to control *M. persicae* in the 1970's also failed, due to unsuitable weather conditions (Remaudiere and Michel, 1971). The history of research into biological control of aphids in glasshouse and field environments has been extensively reviewed in Joshi *et al*. (2010). Limited work has been carried out with regards to biological control of aphids in sugar beet specifically, but this remains an interesting area of

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research and in light of the changes to legislation with regards to neonicotinoids may prove an important tool to the sector in the future.

Moving to a future without neonicotinoid seed treatments, it will be necessary to develop a fully integrated approach to aphid control throughout the UK rotation. It will be critical for the industry to invest in research programmes which study disruptive rotational control of aphids – by species, variety and chemistry – to restrict the proliferation of viruses and ensure the long term durability of existing and future chemistry and resistant and tolerant varieties.

Beet necrotic yellow vein virus

Since the identification of Rhizomania and BNYVV a number of agronomic measures have been used in an attempt to reduce the impact of the disease. A key recommendation is to sow and establish a crop early, particularly in countries where soil temperatures are sufficiently low when the crop is sown. *P. betae* only becomes active and able to transmit the virus at temperatures of 12°C and above (Blunt *et al.,* 1991) whereas the germination and growth of sugar beet takes place at temperatures as low as 3°C. Early sowing can therefore reduce yield losses from the disease significantly, as virus multiplication can be significantly reduced if the fungal vector is delayed (Blunt *et al.,* 1991). It is also recommended to avoid excessive soil moisture in areas where Rhizomania is prevalent and maintain good soil structure and drainage, as high soil moisture stimulates secondary zoospores and root infection. Farmers are advised to avoid planting sugar beet two years in a row in the same field and avoid growing sugar beet in fields where highly virulent BNYVV isolates have been identified.

There is currently no available pesticide effective against *P. betae*, but control of the disease is possible by soil fumigation with chemicals such as methyl bromide or dichloropropane. This method successfully killed the fungal vector and increased sugar yields by up to 6 tonnes/ha and 5 tonnes/ha in the USA and France respectively (Martin and Whitney, 1990; Richard-Molard, 1984). However, re-infestation from the lower layers of the soil can occur during the season and so the land would need treating before every crop. It was therefore considered that the use of the chemicals was uneconomical and too damaging to the environment and was consequently phased out (United Nations Environment Programme (UNEP), 1987).

Interest in biological control methods for disease vectors such as *P. betae* is growing, due to increasing instances of resistance-breaking BNYVV isolates and public concerns over the use of chemicals in the environment generally. Several biological control approaches have been investigated over a number of years, but none have been considered successful enough

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to be commercialised. One approach tested was the incorporation of soil rhizobacterium *Pseudomonas fluorescens* in seed treatments to control *P. betae,* but unfortunately sufficient efficacy was not achieved. In 2001, Resca *et al*., genetically modified a strain of *P. fluorescens* to increase production of an antimicrobial metabolite (2, 4-diacetylphorogucinol) which would control *P. betae* infestation. Unfortunately, the approach was not successful, as despite successful colonisation, control of Rhizomania was not achieved. Another promising area of research is the use of *Trichoderma* spp. species to control *P. betae*, due to their ability to parasitize and degrade the cystori of *P. betae* in *vitro*. D'Ambra and Mutto (1986) showed that *T. harzianum* isolates could parasitise and decompose the resting structure of the disease fungal vector. In a similar study, the bacterium biotypes A and B of *Pseudomonas putida*, reduced the disease fungal vector populations by 23% and 75%, respectively (Aksoy and Yilmazz 2008). Grondona *et al., 2001* tested a commercial formulation of *T. atroviridae*, *T. asperellum* and *T. hamatum* applied as a seed treatment or by irrigation and reported a significant increase in sugar yield of a susceptible cultivar when infested with *P. betae*. Recently, Naraghi *et al*., 2014 carried out a study using *T. harzianum* and *Talaromyces flavus* as an antagonistic fungi to help control *P. betae* populations, and reported that these fungal antagonists are capable of both disease suppression by decreasing *P. betae* populations and promotion of sugar beet growth factors in greenhouse conditions when applied as a soil treatment. Although biological control studies are promising, such approaches are still many years from large scale practical application.

Breeding for aphid resistance and host resistance and/or tolerance

Virus Yellows

Breeding for virus yellows resistance or tolerance dates back to the 1960's with significant efforts being made in Europe. In France, the breeding station Florimond Desprez bred the first diploid and then tetraploid varieties showing tolerance to virus yellows that performed as well as other commercial varieties in the absence of disease (Desprez, 1968). In Germany, the virus yellows tolerant variety, 'Getola' was introduced in 1962 (Kock, 1974; Streudel and Schlösser, 1964) and in Poland, at the 'IHAR' Institute Bydgoszcz, breeders were successful in combining virus yellows tolerance from hybrids between *Beta lomatogona* and tetraploid sugar beet (Pavelska-Kozinska, 1966). The breeding programme at Hilleshög FRO AB, in Sweden, developed lines which combined virus yellows, high yield, sugar content and juice purity. This work resulted in the variety 'Vytomo', however this variety yielded significantly less than control varieties in the absence of disease (Steudel, 1978). In the UK, the variety Maris Vanguard was released in 1966, which was bred at the Plant Breeding Institute in the UK and Broom's Barn Experimental Station. It showed tolerance to a wide range of BYV and BMYV strains and isolates and provided good resistance to Alternaria (Russel, 1965). In the

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absence of yellows root yields of the variety were comparable to other commercial varieties, however sugar and juice purity were low (Russel, 1978).

Although full resistance was not achieved during the breeding efforts of the 1960's and 1970's it was proven possible to reach a significant level of tolerance. But due to the development of neonicotinoid seed treatments controlling aphid vectors in the 1990s, tolerant material was not fully utilised and virus yellows breeding efforts decreased dramatically across Europe. Currently, there are no varieties commercially available which confer tolerance or resistance to virus yellows, posing a major challenge to the sugar beet industry in light of the recent ban on the use of neonicotinoid seed treatments. However, in the UK there has been a sustained prebreeding effort to identify new sources of resistance or tolerance to virus yellows. In 2004, Luterbacher *et al.,* assessed resistance to BYV in 597 Beta accessions collected worldwide and identified a number of highly resistant individual accessions. The resistant plants were then crossed with susceptible sugar beet plants to generate populations for mapping (Francis and Luterbacher, 2003; Luterbacher *et al.,* 2004). In 2008, Grimmer *et al.,* went on to carry out the first genetic mapping analysis of key mapping populations which led to three BYV resistance QTLs mapping to chromosomes III, V and VI. More recently, a 5 yr prebreeding project funded by Innovate UK, The British Beet Research Organisation and UK plant breeders (SESVanderHave and Maribo-Hilleshog) is being carried out at ADAS to identify novel sources of resistance and tolerance to the virus yellows complex. The consortium have identified a number of key resistance and tolerance QTL controlling virus yellows and are currently testing sugar beet hybrids carrying the resistance genes for yield. The five year project will accelerate production of new varieties that provide host protection against the virus yellows complex and ultimately help mitigate the emerging risk of virus yellows to the beet industry since the recent ban on neonicotinoid seed treatments (James *et al.,* 2018; IIRB conference proceedings) and further restrictions on the use of foliar insecticides

Attempts to breed for genetic resistance to aphids started in the 1960s, and resistance to several aphid species was identified which might be species-specific (Russell, 1972; Lowe & Singh, 1985; reviewed by Van Geyt *et al*., 1990). A range of biochemical parameters including the contents of leaf malonic dialdehyde, chlorophyll, carotenoid and total phenolic compounds and the peroxidase activity in seedlings were suggested as parameters for early identification of sugar beet lines for resistance to aphids (Pokhiton & Nechiporuk, 1987; Nechiporuk, 1989). High levels of resistance to *M. persicae* colonisation was found in *B. vulgaris* ssp. maritima (Dale *et al*., 1985). Multiplication of *M. persicae* was reported to be slow on *Beta corolliflora* and low susceptibility to aphids was recorded in species of section *Procumbentes* (reviewed by Van Geyt *et al*., 1990). In 2008, Dewar *et al*., reported that the survival of *M. persicae* (placed on mature leaves in clip cages) declined as the plants aged. They also reported a

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build up of a black deposit in the aphids stomachs, which was thought to be the cause of death. Interestingly, when plants were infected with BYV or BMYV the rate of death and the proportion of aphids dying with balck deposits decreases significantly, when compared to healthy plants. This phenomena is thought to be part of a mature plant resistance mechanism in sugar beet. In 2019, a PhD project began at Wageningen University (funded by IRS, BBRO & SV) to better understand the mechanism of mature plant resistance and increase its applicability to integrated pest management (IPM) strategies. It aims to improve knowledge on how viruses are able to manipulate plants and their insect vectors, with a view to developing a novel breeding strategy for aphid and virus yellows resistance in the future.

Beet necrotic yellow vein virus

Work to develop sugar beet varieties resistant to Beet necrotic yellow vein virus began in the 1970's, but it took more than a decade for the first resistance sources to be developed (reviewed by Biancardi *et al.,* 2002; Scholten and Lange, 2000). Research by the Holly Sugar Company in the USA identified the highly effective 'Holly' source of resistance, which was identified as a single dominant gene mapping to chromosome III, which went on to be named Rz1 (Lewellen *et al.,* 1987). Rz1 confers partial resistance and induces significant resistance to BNYVV multiplication and translocation within the root system, but does not perform well under high disease pressure (Lewellen, 1995; Paul *et al.,* 1993b). The first varieties carrying BNYVV resistance performed better than susceptible cultivars in fields with a low to moderate rhizomania infestation (Winner, 1988), but in fields where no BNYVV was present, a yield penalty associated with the resistance was found. However, over the last thirty years breeders have rapidly improved the agronomic performance (including reducing bolting levels)of resistant cultivars and Rz1 technology is widely used to good effect.

In addition to Rz1, accession WB42 which confers monogenic resistance was found to be more effective than 'Holly' resistance (Paul *et al.,* 1993b) and the major resistance gene (Rz2) also mapped to Ch III at a distinct loci (Scholten *et al.,* 1999; Amiri *et al.,* 2003). Three additional resistances have been identified: Rz3 identified from the WBR1 source (Ginder *et al.,* 2005), Rz4 from the R36 source and Rz5 from the WB258 source (Grimmer *et al.,* 2007). However, detailed mapping analysis determined that only two distinct loci exist, with the first locus represented by alleles Rz1, Rz4 and Rz5 and the second alleles Rz2 and Rz3. Over recent years breeders have begun to combine Rz1 and Rz2 resistance into commercial varieties, producing resistant cultivars which have lower infection levels to resistance-breaking isolates (Liu and Lewellen, 2007). The development of Rz resistance was seen as a major plant breeding breakthrough by the industry and, thirty years later, genetic resistance remains the only effective way to control BNYVV and Rhizomania. However, the presence of

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virulent forms of BNYVV and resistance-breaking isolates suggests that further research is required to identify new sources of resistance for breeders to develop (Heijbroek *et al.,* 1999; Liu *et al.,* 2005). In particular, the long-term reliance on resistant cultivars carrying Rz1 is thought to have encouraged selection of resistance-breaking BNYVV isolates in the USA (Acosta-Leal *et al.,* 2008). To facilitate the search for novel BNYVV resistance genes, Fan *et al.* (2015) carried out a transcriptome analysis of infected and uninfected *Beta macrocarpa* plants. The study revealed 261 genes that were differentially expressed in infected plants compared to control plants. More than 50% of the differentially expressed genes were involved in biotic stress and wound responses, reactive oxygen species (ROS) metabolism, pathogenesis-related (PR) infection proteins, transcription factors and putative disease resistance genes. This study represents the first application of Illumina sequencing technology to obtain transcriptome data of the infection pathway of BNYVV in wild beet species, in order to accelerate research on the infection mechanism of BNYVV in sugar beet and aid the identification of novel resistance genes for future cultivars. If durable sources of BNYVV resistance cannot be identified in the short-medium term, alternative approaches to controlling the virus may be necessary in the future.

Polymyxa betae

An alternative breeding approach is to develop resistance to *Polymyxa betae*, as a means of preventing or reducing entry of the virus. Resistance to *P. betae* has been studied in wild *Beta* species and shown to reduce or eliminate BNYVV transmission in *Procumbentes* (Paul *et al.,* 1992b; Barr *et al.,* 1995) and *Corollinae* (Paul *et al,* 1993b). *P. betae* zoospores have been observed to attach to and penetrate the roots in these wild species with a similar frequency as in susceptible sugar beet, but the plasmodiophorid rarely develops further, resulting in many fewer *P. betae* resting spores in their roots. As well as significantly reducing levels of *P. betae* in the roots, there was also a reduction in the level of BNYVV (Paul *et al.,* 1992b). Rare monosomic addition lines of sugar beet carrying individual chromosomes of either *B. patellaris* or *B. procumbens* were used to demonstrate that genes conferring the *P. betae* resistance phenotype were located on chromosomes IV and VII of both wild *beta* species (Mesbah *et al.,* 1997; Paul *et al.,* 1992b). A reduction of virus levels was also observed in the addition lines. Unfortunately, due to incompatibility of both wild species with sugar beet, accessing this form of resistance through conventional breeding methods has not been possible (Van Geyt *et al.,* 1990). However, partial resistance was also identified in an accession of wild sea beet compatible with sugar beet (Asher and Barr, 1990) and further QTL mapping analysis led to the identification of two interacting quantitative trait loci associated with *P. betae* resistance, located on chromosomes IV and IX (Asher *et al.,* 2008). As yet, no *P. betae* resistant varieties are commercially available.

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Genetic modification and CRISPR technology

Since the 1990's there has been a range of transgenic resistance approaches carried out to induce resistance to BNYVV in transgenic sugar beet. Mannerlöf *et al.* (1996) generated transgenic sugar beet which expressed the BNYVV coat protein (CP) sequence and subsequently reported significantly reduced levels of virus in glasshouse and field environments. This study was followed in 1997 by a transgenic approach which used the expression of a short-chain antibody fragment (scFv) that was specific to the BNYVV CP in *N. benthamiana*. Plants expressing the BNYVV CP-specific scFV showed a delay in the build-up of detectable BNYVV levels in the leaves following mechanical inoculation or viruliferous *P. betae* inoculation. However, later in the cycle, virus levels in the transgenic plants were comparable to the control lines (Fecker *et al.,* 1997). Jafarzade *et al.* (2019) took a similar approach using a cDNA encoding a scFv fragment specific to the main BNYVV coat protein (P21). The cDNA was targeted to the cytosol, appoplast and mitochondrial outer membrane of *Nicotiana benthamiana* plants. It was reported that the titre of virus was also significantly reduced when exposed to BNYVV-infested soil. In addition, Andika *et al.* (2005) expressed the CP read-through domain ORF of BNYVV in *N. benthamiana*, and showed that transgenic plants had very low levels of virus following inoculation with the virus due to RNA silencing. A similar approach was taken by Lennefors *et al.* (2006) whereby an inverted cDNA repeat of 0.4kb derived from the BNYVV replicase gene was transformed into sugar beet to induce resistance via RNA silencing. Transgenic plants displayed high resistance to BNYVV A-, B- and P- types when inoculated with *P. betae* and virus titres in the transgenic plants were significantly lower than in the resistant 'Holly' carrying Rz1. Resistance was not overcome by co-infection with four other common sugar beet-infecting viruses from the genera *Pomovirus*, *Polerovirus* and *Closterovirus* suggesting the resistance phenotype will be durable in the field (Lennefors *et al.,* 2008). Jiang *et al.* (2019) has developed a BNYVV infectious cDNA clone and engineered a set of BNYVV-based gene expression vectors with four insertion site vectors that can express recombinant proteins in the model *Nicotiana benthamiana* and sugar beet plants. These vectors can be used to investigate the subcellular co-localisation and function of multiple proteins in leaf, root, and stem tissues of systemically infected plants. They also demonstrated that BNYVV-based vectors can be used to deliver guide RNA for CRISPR/Cas 9 plant genome editing. This novel molecular approach provides a powerful and efficient platform for expression of multiple genes and for functional characterisation of genes in future studies, in a plant genus that is not easily transformed (Jiang *et al.,* 2019). Transgenic control of BNYVV remains an appealing alternative to traditional plant breeding, particularly in areas with resistance-breaking strains or extremely high disease pressure.

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Discussion

The discussion of this review is presented in the form of:

i) A summary of the major virus diseases affecting arable and vegetable crops in the UK, their vectors and alternate hosts and their primary methods of detection.

ii) A prioritised list of control options, both applied and fundamental which should be investigated and/or applied to UK production systems.

iii) Recommendations to inform research and knowledge exchange on virus management. .

Table 42. A summary of the major virus diseases affecting cereals, oilseeds and sugar beet crops in the UK, their vectors and alternate hosts, their primary methods of detection and available facilities in the UK to carry out detection.

Crop	Key virus(es)	Key vectors	Key alternate hosts	Seed testing	Detection method available?	Testing facility
Potato	PVY	*M. persicae* *A. pisum* *R. padi*	Over 60 different plant species including 14 genera of Solanaceae	No	ELISA	NIAB FERA SASA Tests available from Pocket diagnostics and Lynchwood diagnostics
	PLRV	*M. persicae* *M. euphorbiae* *A. solani*	Over 30 different plant species	No	ELISA	NIAB FERA SASA Tests available from Lynchwood diagnostics
Cereals	BYDV	*R. padi* *M. dirhodum* *R. maidis* *S. avenae*	Over 30 different species	No	ELISA PCR LAMP	NIAB FERA Tests available from Lynchwood diagnostics
	BaMMV	*Polymyxa graminis*	Hare's tail grass (*Lagurus ovatus*)	No	ELISA PCR RFLP	FERA Tests available from Lynchwood diagnostics
	BaYMV	*P. graminis*	None	No	ELISA PCR RFLP	NIAB FERA Tests available from

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						Lynchwood diagnostics
	SBCMV	*P. graminis*	Meadow brome Rye	No	ELISA PCR	NIAB FERA
Oilseed rape	TuYV	*A. craccivora* *A. gossypii* *A. solani* *B. helichrysi* *B. brassicae* *M. euphorbiae* *M. ornatus* *M. persicae*	Over 50 different Brassica and dicotyledonous species	No	ELISA	FERA NIAB
Sugar beet	BNYVV	*Polymyxa betae*	*Gomphrena globose* *Beta macrocarpa* *Chenopodiaceae*	No	ELISA RT-PCR	BBRO FERA Tests available from Lynchwood diagnostics
	BYV, BMYV, BChV	*M. persicae* *A. fabae*	*Chenopodiaceae* *Amaranthaceae* *Aizoaceae* *Caryophyllaceae* *Tetragonia* spp. *Nicotiana* spp.	No	ELISA RT-PCR	BBRO FERA for BYV Tests available from Lynchwood diagnostics

Table 43. A summary of the major virus diseases affecting UK field vegetable and protected tomato crops, their vectors and alternate hosts, primary methods of detection and available facilities in the UK to carry out detection.

Crop	**Key viruses**	**Key vectors**	**Key alternate hosts**	**Seed testing**	**Detection method available?**	**Testing facility**
Root crops	PYFV	*C. aegopodii* *C. pastinacae*	Cow parsley Hogweed Chervil Spinach *Chenopodiaceae* *Nicotiana* spp.	No	ELISA PCR	
	CMD	*C. aegopodii*	Common bean Coriander *Chenopodiaceae* Chervil *Nicotiana* spp.	No	PCR	FERA
	CaTV	*C. aegopodii*	Chervil	Possible	PCR	FERA

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Crop	Key viruses	Key vectors	Key alternate hosts	Seed testing	Detection method available?	Testing facility
		M. persicae	*Nicotiana* spp.			
	CYLV	*C. aegopodii* *M. persicae*	Chervil Coriander *P. anisum* *Nicotiana* spp.	No	PCR	FERA
Peas	PSbMV	*M. euphorbiae* *M. persicae* *A. pisum* *A. craccivora* *A. fabae* *R. padi*	Lentil Chickpea Broad bean Shepherd's purse Black medic Alfalfa *Chenopodiaceae*	Yes	ELISA	NIAB FERA Tests available from Lynchwood diagnostics
	BLRV	*A. pisum* *A. craccivora* *M. persicae*	Alfalfa, White clover Broad bean Common bean Chickpea Cowpea Lentil	No	ELISA	FERA Tests available from Lynchwood diagnostics
	BYMV	*A. pisum* *M. euphorbiae* *M. persicae* *A. fabae*	Common bean, *Gladiolus* spp. *Freesia* spp. Soybean. *Trifolium* spp. Broad bean Alfalfa	No	ELISA	FERA Tests available from Lynchwood diagnostics
Vegetable Brassicas	TuYV	*A. craccivora* *A. gossypii* *A. solani* *B. helichrysi* *B. brassicae* *M. euphorbiae* *M. ornatus* *M. persicae*	Wide host range in over 50 different Brassica and Dicotyledonous species	No	ELISA	FERA
Cucurbits	CYSDV	*B. tabaci*	*Cucurbit* spp. *Cucumis melo* L. Lettuce Snap bean	No	ELISA	FERA Tests available from Lynchwood diagnostics

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Crop	Key viruses	Key vectors	Key alternate hosts	Seed testing	Detection method available?	Testing facility
	CMV	*A. pisum* *A. craccivora* *M. persicae*	CMV infects over 1200 plant species	No	ELISA PCR	FERA Tests available from Lynchwood diagnostics
	WMV	*M. persicae* *A. solani* *A. craccivora* *M. euphorbia*	WMV infects over 170 plant species	No	ELISA	FERA Tests available from Lynchwood diagnostics
	MWMV	*A. gossypii* *A. craccivora*	*C. lanatus,* *Cucumis* spp. *C. pepo* *M. parviflora*	No	ELISA	Tests available from Lynchwood diagnostics
Lettuce	Big vein disease	*O. brassicae*	*Chenopodiaceae* *Nicotiana* spp., *Sonchus* spp.,	No	ELISA	FERA Tests available from Lynchwood diagnostics
	LMV	*A. gossypii* *M. euphorbiae* *M. gei* *M. persicae*	LMV infects over 100 plant species	Yes	ELISA	NIAB FERA
	LNYV	*H. lactucae* *H. carduellimus* *B. tabaci*	*L. esculentum* *Nicotiana* spp. *Sonchus* spp. Spinach	No	Monoclonal antibodies developed by Warwick University	
Alliums	OYDV	*M. persicae* *R. maidis* *A. pisum*	*Chenopodiaceae*	No	ELISA	FERA
	IYSV	*T. tabaci* *F. fusca* (Hinds)	IYSV has a broad host range	No	ELISA PCR	FERA Tests available from Lynchwood diagnostics
Asparagus	AV-1	*A. craccivora* *M. persicae*	*A. tuberosum* *Chenopodiaceae* *G. globose* *T. tetragonioides*	No	ELISA	FERA Lynchwood diagnostics
	AV-2	No known vector	*B. vulgaris* *Chenopodiaceae*	Yes	ELISA	FERA Tests available from

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Crop	Key viruses	Key vectors	Key alternate hosts	Seed testing	Detection method available?	Testing facility
			C. sativus *G. max* *G. globose* *Nicotiana* spp. *O. basilicum* Common bean *V. unguiculata*			Lynchwood diagnostics
Protected tomato	PepMV	Wide range of transmission routes	Wide range of Solanaceae hosts	Yes	ELISA PCR	FERA Tests available from Lynchwood diagnostics
	TSWV	*F. occidentalis* *F. fusca* (Hinds)	Over 86 botanical families	No	ELISA PCR	FERA NIAB Tests available from Lynchwood diagnostics
	ToMV TMV	Wide range of transmission routes	Wide host range, affecting over 40 botanical families	Yes (ToMV)	ELISA and PCR (ToMV) ELISA (TMV)	FERA Tests available from Lynchwood diagnostics

*Note: Both ADAS and STC offer a virus testing service across multiple crops, but this is done on a case by case basis according to the client needs.

Table 44. A prioritised list of control options, both applied and fundamental which should be investigated and/or applied to UK production systems.

Monitoring and thresholds	
Crop	**Method**
All crops	Knowledge exchange to inform growers and advisors on how best to detect for viruses and avoid misdiagnosis.
	Development of health and safety and legislation frameworks for drone usage.
	Monitor for emerging cases of resistance in UK aphid species covering changes in resistance levels and new cases of resistance.
	Monitor for arrival of non-indigenous aphid species vectoring new viruses or virus strains.
	Develop further NGS methods for identification of new and unknown viruses outside the scope of those already being investigated.
	Further develop hyperspectral imaging methods to identify viral infection

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Sugar beet,	Development of a high through put in field bioassay for virus yellows detection using a Bioelectric Recognition Assay (BERA-HTP) or LAMP assay. Development of high through-put qRT-PCR methods for persistent and semi-persistent yellows viruses e.g. field collected aphids, host canopy and tap roots to understand proportion of aphids carrying the individual viruses and geographical spread within crops. Update pesticide thresholds for control of virus yellows and develop improved decision support systems (DSS).
Cereals & oilseed rape	Improve monitoring methods for BYDV and TuYV vectors. This should include schemes that determine the proportion of vectors carrying the virus, remote sensing, image analysis for aphid identification and novel molecular diagnostics.
Cereals	Develop improved decision support systems (DSS) for the management of BYDV. This should include understanding of the impact of the proportion of aphids carrying BYDV on yield and management decisions.
Potato	Update predictive models so they are capable of predicting aphid migration and virus risk in a changing environment. Develop high throughput field bioassay for PVY isolates and PLRV e.g. Bioelectric Recognition Assay (BERA-HTP) methods, LAMP assay.
Oilseed rape	Develop models to better predict infection, spread and yield impact of TuYV. These should then be developed into DSS to assist in management of the virus.
Root crops	Perform baseline surveys on the prevalence of PYFV, CYLV and CaTV in the UK.
	Further investgate the relationship between numbers of willow-carrot aphid trapped and the amount of damage to the crop if left untreated.
	Establish the potential for carrot viruses to be seed transmitted.
Peas	Expand on the number of viruses which can be tested for through commercial seed testing services.
	Further develop molecular assays for detection of pea specific viruses.
	Monitor for the presence of the newly emerging group of Nanoviruses in the UK, particularly PNYDV.
	Adapt and develop the model used for forecasting PSbMV in Australia for use under UK climatic conditions.
Vegetable Brassicas	Knowledge exchange activities to improve grower and advisor awareness around monitoring for virus symptoms before heads go into storage.
	Explore the potential for using epidemiological modelling of TuVY in vegetable Brassicas.
Lettuce	Establish which pathotypes of LMV are present in LMV infected lettuce in the UK.
	Further investigate possible additional vectors and alternative hosts for LNYV.
	Develop immuno-based assays for LNYV, if none commercially available already.

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Alliums	Develop a monitoring service for OYDV and IYSV which both have the potential to become established in Allium crops such as onion and leek in the UK
Asparagus	Develop a seed testing service for AV-2 so that seed can be virus-indexed before purchase,
	Further develop decision support systems and prediction modelling for aphid control in minor use crops such as asparagus.
Tomatoes	Improved ToBRFV detection, including a rapid species specific diagnostic test e.g. LFD kit, indicator plants etc.
	Knowledge exchange of the symptoms of emerging viruses, including ToBRFV to all members of the tomato industry, especially crop workers.
Cultural controls and hygiene	
All crops	Increased research into disruptive rotational control of aphids – by species, variety and chemistry
	Investigate alternative cultural control methods such as mineral oil application and crop borders to prevent virus spread.
Peas	Establish the potential for new legume crops being introduced into the UK to act as potential sources of new viruses and alternate hosts of current pea viruses.
Field vegetables	Evaluate the potential for use of mulches (recyclable plastic, woodchip or straw) as control method for weeds as alternate, and the effect on the crop.
Vegetable Brassicas	Explore the potential of using different types of netting to exclude small and larger aphids, and its impact financially as well as its effect on the microclimate of the crop.
	Investigate the use of intercropping with catch crops such as shallots for management of cabbage aphids.
Cucurbits	Establish the routes e.g. through plant propagators, alternative trade pathways through which Cucurbit viruses enter the UK.
	Establish and enforce better plant health and quarantine standard to prevent new viruses of Cucurbits entering the UK.
Varietal resistance	
All crops	Review the ethics and regulations surrounding the use of gene editing and CRISPR technology in the UK.
Cereals	Develop varietal resistance/tolerance to BYDV in wheat.
Sugar beet	Continued development of durable host resistance/tolerance to the virus yellows complex (BYV, BMYV, BChV). Development of varieties with host resistance to *Myzus persicae* Identification of novel BNYVV resistance sources to complement Rz1/Rz2 technology to provide protection against resistant breaking strains.
Field vegetables	Examine the potential for resistant varieties developed abroad to be grown in under UK climatic conditions.
Vegetable Brassicas	Determine the resistance/tolerance status of currently available vegetable Brassica varieties through creation of independent trials data.

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Tomato	Development of resistant varieties to ToBRFV, PepMV, STV, ToCV, TICV and those resistant to the new SW5 resistance breaking strain of TSWV.
Cucurbits	Exploit sources of genetic resistance and tolerance already available in other Cucurbit spp. and their feasibility for transfer into commercially grown courgette.
Vector and viral biology	
All crops	Inform growers on all potential routes for aphid infestation and issue guidance for control
Sugar beet	Updating research on the risk of virus yellows spread via alternative hosts (weeds, wild and cultivated beet including fodder, red beet, energy beet) across the UK, either by acting as a viral pool or by encouraging overwintering of aphid vectors.
Cereals and oilseed rape	Improved understanding of how aphids locate crops and whether this can be used in BYDV and TuYV management.
Carrot	Establish the relationship of the association of symptoms with CMD infection
	Investigate the biological properties of CaTV to allow for the development of immuno-based diagnostic assays.
Cucurbits	Investigate the potential for MWMV to be whitefly transmitted
Tomato	Establish the longevity of ToBRFV on different surfaces and within the endosperm and if it is spread via the irrigation system/nutrient feed solution.
	Identify alternative host species of ToBRFV and other viral issues
	Further research into STV to establish pathogenicity, or the requirement for mixed infections for symptom development.
Biological and alternative control methods	
All crops	Research and KE activities into habitat management to improve natural enemy activity.
	Further investigate the potential of biopesticides for control of aphid vectors under field conditions.
Cereals and oilseed rape	Develop a better understanding of the impact of natural enemies have on controlling BYDV and TuYV vectors.
Tomato	Development of mild viral strains, similar to the PepMV®-01, to vaccinate against ToBRFV.
	Identify new/existing WFT and whitefly controls (chemical/biological or technological) for use in protected tomato
Chemical control	
All crops	Investigate the use of adjuvants or other substances to improve efficacy of currently available insecticides and increase crop safety.
	Development of novel active ingredients which can deliver speed of knockdown for controlling transmission of semi-persistent viruses.
	Investigate improved resistance management strategies for insecticide resistance in key virus vectors.

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Field vegetables	Investigate how market driven perfection of products drives the use of insecticides as control options and how this could be changed to increase the use of IPM
Vegetable Brassicas	Continue to evaluate new actives which are being developed for oilseed rape in vegetable Brassicas to widen the range of actives available.
Tomato	Identify effective disinfectants, on a selection of different surfaces, for effectively eliminating ToBRFV.

Recommendations to inform research and knowledge exchange.

Improve awareness of potential viral threats: The review identified a large number of potential viruses which could threaten crops in the UK in the future. Factors such as climate change and importing of plants from outside of the UK could result these viruses becoming established in the UK.

Increase access to, and the use of, current knowledge: There is more information on virus management and vector control known than is readily available to advisors and growers. Using current knowledge better is a very high priority. For management of vectors which affect multiple crops such as *M. persicae*, a knowledge hub that is irrespective of crop sectors should be developed to provide simple messages for growers and advisors.

Reduce the gaps between practical knowledge and fundamental research: There is too great a gap between applied knowledge generated for growers and fundamental research at an academic level. More knowledge exchange activities including hands on workshops, crop walks and open days e.g. on Strategic Farms will ensure fundamental research translates into tangible changes in practices.

Maximise pesticide availability: Good stewardship of current active substances is vital. Companies, regulators and users need to work together to develop new actives and retain old actives through establishing and supporting best practice guidelines.

Develop a better understanding of application and management of biopesticides: As the use of biopesticides increases, it is important that biopesticides are not used as a simple like-for-like replacement for conventional chemical pesticides. Further work and knowledge exchange activities on the use of biopesticides in field cropping systems is required.

Agree funding for an integrated approach to virus management: There is a strong social and political desire to maximise non-chemical and more integrated approaches. Government and industry need to working together to achieve this.

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Research into management of viruses needs to be considered more strategically: The current approach to virus management is very often based on specific viruses and/or in specific crops. A more strategic approach to virus management over the whole cropping system is needed, particularly for agnostic insect vectors such as *M. persicae* and *B. tabaci* and virus species such as CMV and TuYV which affect multiple crops in arable and horticulture. This approach should cover the four basic principles of virus management;

- Start with clean material
- Grow in the absence of vectors and alternate hosts
- Crop rotations and isolating from similar crops
- Exploit available varietal resistance and tolerance

Knowledge and Technology Transfer

The review has been discussed with a wide range of growers, agronomists, breeders and scientists. This report will be disseminated to all relevant levy payers, appropriate research organisations and contractors.

Glossary

BLAST-Basic Local Alignment Search Tool

bp-base pair

CP-coat protein

DBIA-dot blot immunoassay

DNA- deoxyribonucleic acid

DAS-ELISA- double antibody sandwich enzyme-linked immunosorbent assay

ELISA- enzyme-linked immunosorbent assay

Kb-kilo base pairs

NGS-next generation sequencing

nt-nucleotide

PCR-polymerase chain reaction

RNA-ribonucleic acid

RT-PCR-real time polymerase chain reaction

TBIA-tissue blot immunoassay

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