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Project leader:	Sarah Mayne, ADAS UK Ltd
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Key staff:	Tim O'Neill, ADAS UK Ltd Matt Dickinson, University of Nottingham; Bethan Warman, University of Nottingham Anna Skelton, Fera Science Ltd
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Industry Representative:	Dr Phil Morley, APS Salads/TGA Brian Moralee, Wight Salads, Main Road, Arreton, Isle of Wight
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

Sarah Mayne

Plant Pathologist

ADAS UK Ltd

Signature ..... Date .....

Bethan Warman

MRes student

University of Nottingham

Signature ..... Date .....

Report authorised by:

Barry Mulholland

Head of Horticulture

ADAS UK Ltd

Signature ..... Date .....

Matt Dickinson

University of Nottingham

Signature ..... Date .....

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GROWER SUMMARY

Headline

- Mixed strain infections of PepMV are present in some UK tomato crops
- Overall, mixed infections, especially those including the US1 strain, were associated with more severe fruit symptoms, and care should be taken to avoid introduction of additional strains of PepMV even on sites where infection has already been confirmed
- The risk of PepMV transmission from small root pieces that remain in soil after crop removal appears to be very small
- Irrigation can be a source of PepMV, depending on water sources utilised

Background

Pepino mosaic virus (PepMV) is one of the most economically important diseases of tomatoes in the UK. In 2013 and again in 2014, crops of Piccolo on several nurseries were severely affected with three-four and occasionally more trusses of fruit affected, resulting in substantial financial losses. Symptom severity in the same variety can vary greatly on different nurseries, raising the question of possible mixed strain infections. Efforts to exclude the virus from a nursery and prevent persistence between successive crops by strict hygiene measures have had limited success. LAMP assays that can discriminate CH2, EU and US1 strains of PepMV were recently published. The aim of this joint ADAS/University of Nottingham/Fera project was to establish the LAMP assays in the UK so that rapid on-site testing of tomato plants for three different strains of PepMV is possible. Presently, five strains of PepMV are described, though it is the CH2 strain that is prevalent in the UK, with less frequent reports of EU and US1 strains. The additional strains of LP (original Peruvian) and PES (new Peruvian) do not commonly occur in Europe and were not included in this project. The assays were then used to: i) investigate occurrence of mixed-strain infections in crops, especially any with severe symptoms; ii) investigate efficacy of hygiene measures after crop removal in removing PepMV from glasshouse structures and equipment; iii) determine survival in tomato roots in soil and in composted tomato waste. A method for rapid detection of PepMV in water was also examined.

The overall aim of this work was to increase understanding of PepMV symptom severity, persistence on nurseries and mild strain cross-protection. Specific objectives of the project were as follows:

1. To validate published LAMP assays for rapid detection of CH2, EU and US1 strains of PepMV;
2. To determine occurrence of mixed strain PepMV infections in tomato crops;
3. To monitor greenhouse structures and equipment for occurrence of PepMV after crop removal;
4. To determine survival in tomato roots in soil and in composted tomato waste;
5. To validate a method for detection of PepMV in water and test some water samples from UK tomato nurseries;
6. To monitor spread of mild-strain CH2 PepMV and check for other strains in crops inoculated with mild-strain for cross-protection;
7. To communicate results to growers.

It was not possible to investigate Objective 6 over the course of the project as no mild strain *Pepino mosaic virus* was approved for UK crops. However, a CH2 mild strain gained approval in spring 2016 and it is planned to undertake Objective 6 in a follow-on project in 2017.

Summary

Objective 1 – Validate published LAMP assays for rapid detection of CH2, EU and US1 strains of PepMV

Primers that had been validated in previous studies overseas were purchased and used in RT-LAMP assays to test samples for the presence of PepMV. There were three sets of primers, one each for the CH2, EU and US1 strains of PepMV infection, allowing for the identification of the specific strain of PepMV present in an infected sample. Initial RT-LAMP tests on tomato samples from Sutton Bonington confirmed that the CH2 primer was successful. In order to validate the EU and US1 primers, purified RNA extracts of the EU and US1 strains were obtained from Fera Science Ltd. After confirming that each primer was successful in detecting its respective PepMV strain, the primers were used in RT-LAMP assays to test tomato fruit and leaf samples, swab samples, compost samples and water samples for the presence of PepMV. By testing samples with all three sets of primers, it was possible to determine whether or not mixed-strain infections were present in the samples. In samples from a tomato crop found to be infected with all three strains, the specificity of the LAMP assays was confirmed when it was shown that the CH2, EU and US1 tests had different

annealing temperatures (the temperatures at which specific primers attach to amplify DNA sequences). Results from these tests provided an insight into the distribution and presence of mixed-strain PepMV infection in tomato crops in the UK and provided information of sources of PepMV inoculum within the glasshouse.

Objective 2 – Determine occurrence of mixed strain PepMV infections in tomato crops

The variety of symptoms recorded throughout the projects can be seen in Figure 1. Details of the crops visited and the times of year these visits occurred are summarised in Table 1, below. Where possible, crops were each visited twice in the spring or autumn, periods when symptoms of PepMV are usually most obvious and severe.

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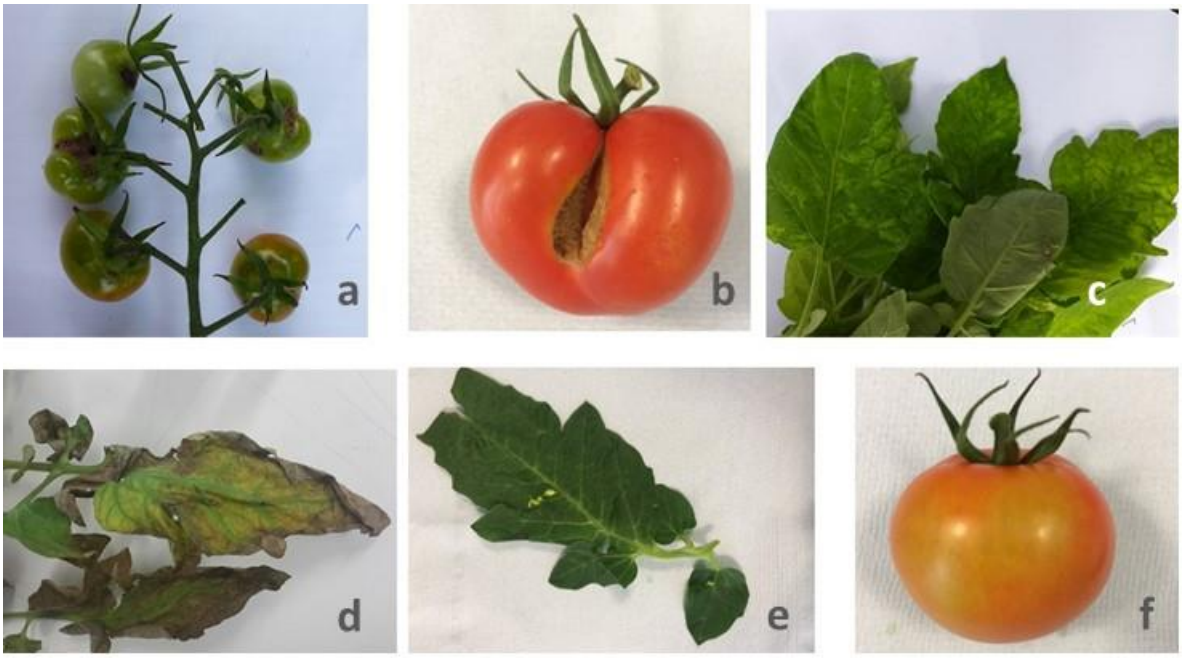

Figure 1. Examples of symptoms observed associated with PepMV infection including splitting of fruit (a, b), chlorotic heads (c), leaf necrosis (d), chlorotic leaf spotting (e), and fruit marbling (f) – 2015 & 2016.

Table 1. Detail of the six individual tomato crops visited and assessed over 2015 and 2016 for symptoms of PepMV and the strains confirmed on each

Crop	Dates visited	Variety / Scion	Substrate	PepMV strains		
				CH2	EU	US1
1	09/09/2015 07/10/2015	Piccolo / Maxifort	Organic	✓ ✓	- -	- -
2	18/09/2015 12/10/2015	Roterno / Maxifort	Rockwool	✓ ✓	- -	✓ ✓
3	15/10/2015	Piccolo / Maxifort	Rockwool	✓	✓	✓
4	08/03/2016 12/04/2016	Piccolo / Maxifort	Organic	✓ ✓	- -	- -
5	15/03/2016 20/04/2016	Sunstream / Maxifort	Rockwool	✓ ✓	- -	- -
6	08/06/2016	Piccolo / Emperador	NFT	✓	✓	✓

The majority of UK infections monitored appear to be due to the CH2 strain of PepMV, known to be prevalent in Europe currently. Many crops sampled across 2015 and 2016 had only the CH2 strain present on site, the EU strain was also detected in two crops, and three sites tested positive for US1 (detected more often in symptomatic tissue/plants), in addition to both CH2 and EU. Overall, mixed infections, especially those including the US1 strain, resulted in more severe symptoms (Figure 2). Severe fruit symptoms appear more common in large vine varieties than on the variety Piccolo, where fruit symptoms are more likely if a mixed infection is present.

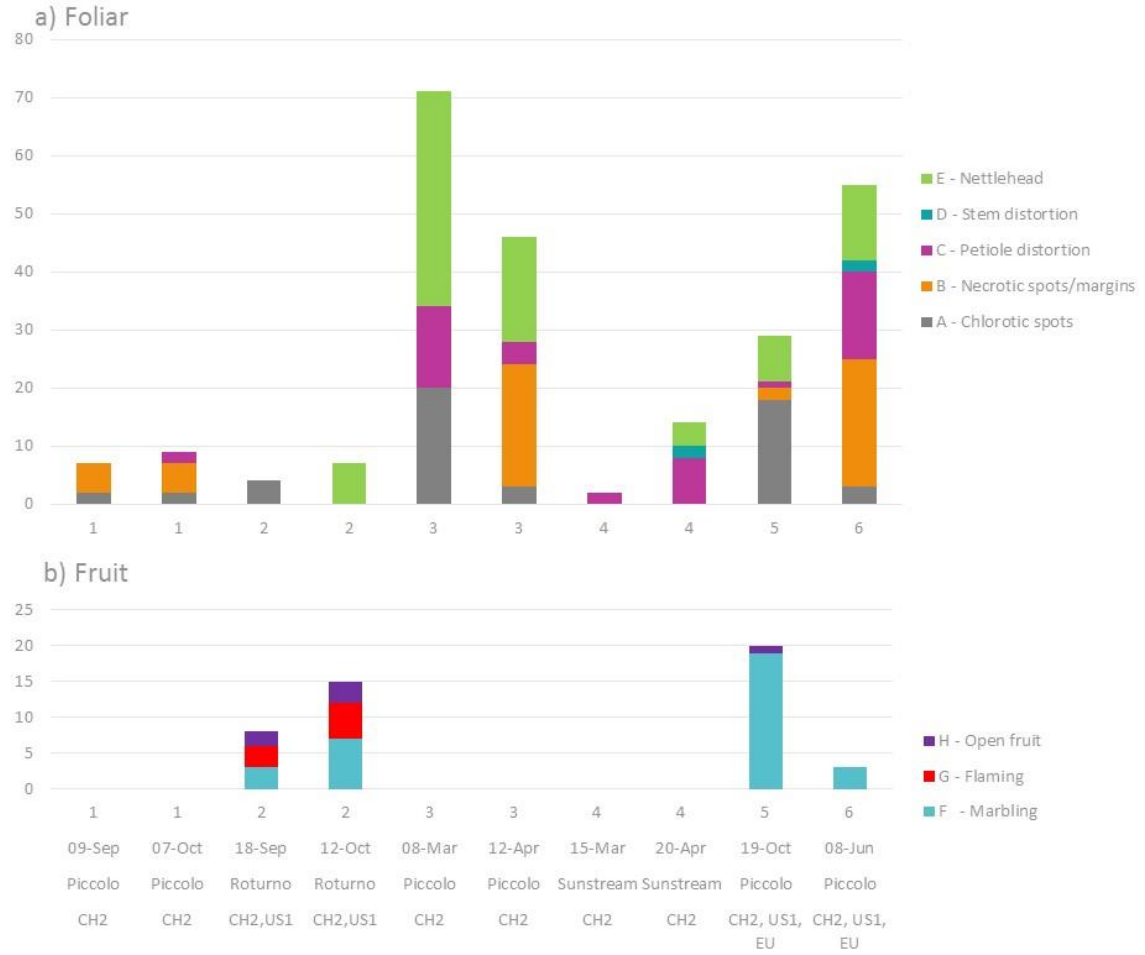

Figure 2. Occurrence (% plants) and type of severe PepMV symptoms (index >2) in tomato crops examined at six UK sites in 2015-2016, a) foliar, b) fruit. The crops at sites 1-4 were each assessed twice, approximately 1 month apart. 100 plants assessed for symptoms and 30 plants tested for PepMV at each visit. Strain of PepMV was determined by LAMP assay.

Objective 3 – Monitor greenhouse structures and equipment for occurrence of PepMV after crop removal

Three commercial sites were visited in autumn 2015, and 50 swabs per site were taken in an area of glasshouse where PepMV infection had been confirmed. A variety of surfaces were swabbed, including glass, plant supports, concrete pathways and electrical boxes. Post clean-up and disinfection, the sites were visited again and the same locations were swabbed. The disinfection process at each site was also recorded, with some sites utilising a more complete clean-up process than others (Table 2).

Table 2. Detail of clean-up protocols used on three tomato nurseries - autumn 2015

Site		Clean-up protocol	
	Crop debris	Cleaning	Biocides
Site 1	All removed as far as possible	Glass and irrigation cleaned. Steam cleaning of structure, equipment and staff clothing cleaned	Sanprox P (peroxyacetic acid, hydrogen peroxide & acetic acid) used extensively. Structures wiped with hypochlorite.
Site 2	All removed as far as possible	Glass and irrigation cleaned	Virkon S (peroxygen compounds) on glasshouse structures, floor. Sanprox P on upper structure.
Site 3	Remains in house, covered in plastic	Glass and irrigation cleaned	Mid-structure sprayed with Horticide (glutaraldehyde + quarternary ammonium compounds)

Before clean-up, as may be expected, PepMV was confirmed on a large number of swabs at all three sites. Following clean-up, the number of swabs testing positively for PepMV fell for all sites, though less so for the crop with the less intensive clean-up process (Site 3). In addition, the time between pre- and post-clean-up visits was approximately 6 weeks for Sites one and two, but was only two weeks for Site 3, which may also account for the greater number of positive results after clean-up at this site. Areas that were in close contact with the crop, and areas that were difficult to disinfect fully such as electrical fuse boxes, were common sites for PepMV detection after clean-up. Swabs were tested by LAMP assay, and a selection of positives were also tested by the sap inoculation method at Fera. Following sap inoculation, none of the swabs taken after clean-up resulted in confirmed infection in tomato seedlings. This implies that the clean-up processes at all three sites were sufficient to remove viable virus, though it is possible viable virus persisted in some areas not swabbed.

A summary of the sites where swab samples tested positive and negative for PepMV by the LAMP assay, before and after disinfection, is shown below (Table 3).

Table 3. Summary of glasshouse swab samples from three nurseries testing positive for PepMV by LAMP assay and the effect of clean up/disinfection between crops (see Appendix 1 for full details)

Area swabbed	Proportion of samples positive for PepMV before (pre) and after (post) disinfection									
	Site 1			Site 2			Site 3			Total
	Pre	Post		Pre	Post		Pre	Post		Pre	Post
Glasshouse door	3/3	1/3		4/4	0/4		3/3	1/3		10/10	2/10
Concrete path	2/2	0/2		4/4	3/4		2/2	0/2		8/8	3/8
Glass wall	0/4	0/4		2/3	0/3		2/2	0/2		4/9	0/9
Mypex/plastic floor	NT	NT		2/4	1/4		1/1	0/1		3/5	1/5
Aluminium stanchion	3/3	0/3		2/2	1/2		3/3	2/3		8/8	3/8
Gutter	NT	NT		NT	NT		2/2	0/2		2/2	0/2
Support wire	1/1	0/1		NT	NT		NT	NT		1/1	0/1
Irrigation line/drip line	3/5	0/5		0/2	0/2		4/4	4/4		7/11	4/11
Drip peg	3/3	0/3		2/2	0/2		2/2	2/2		7/7	2/7
Heating pipe/metal	6/6	0/6		2/3	2/3		2/2	1/2		10/11	3/11
Heating pipe supports	2/2	1/2		2/3	2/3		4/4	4/4		8/9	7/9
Trolley/truck	5/5	4/6		10/10	6/10		5/5	3/5		21/21	13/21
Picking crate	3/3	0/3		3/3	1/3		3/3	2/3		9/9	3/9
Electric box/switch	2/3	1/3		2/3	2/3		6/6	6/6		10/11	9/11
Waste bin	1/1	0/1		1/1	1/1		1/1	1/1		3/3	2/3
Hand sanitiser	0/1	0/1		NT	NT		2/2	2/2		2/3	2/3
Water cooler	3/4	1/4		1/1	0/1		1/2	0/2		5/7	1/7
Other	1/3	1/3		5/5	2/5		6/6	4/6		12/14	7/14
Total	32/50	9/50		42/50	21/50		49/50	32/50		122/150	62/150

NT – not tested

Objective 4 – Determine survival in tomato roots, in soil and in composted tomato waste

Survival of PepMV was investigated by pulling out plants in a commercial organic crop, and sampling roots left behind in the soil for 6 weeks following plant removal. Roots were loosened before pulling out so that most roots of large and medium thickness were removed. Six weeks is slightly longer than the period normally left between removal of one crop and planting of the next crop at this site. Five thick and five thin roots were sampled every two weeks, and sent to Fera to be tested by ELISA and subsequently by sap inoculation of tomato seedlings and *Nicotiana benthamiana* plants to check for viability of the virus. This investigation was carried out in summer, rather than in autumn/winter as would occur commercially. Soil temperatures ranged between 23 and 27.1°C, whereas temperatures closer to 18°C might be expected at the usual time of year.

Over the course of sampling, a trend for a reduction in detectable PepMV was observed when tested by ELISA (Table 4). Results also showed that PepMV was recovered more often from thick roots than thin roots, though at T6 this trend was not conserved. By the final sampling date, 6 weeks after plants had been pulled out, finding roots of appropriate size proved difficult, and the roots sent for sampling at this point were smaller in diameter than those sampled earlier in the process.

Table 4. Detection of PepMV by ELISA and detection of viable PepMV by transmission test in root pieces sampled from soil at intervals (0-6 weeks) after plant removal

Sample time	Rep	No. roots positive (of 5)		No. positive roots shown to have viable PepMV
		Thick (≥5 mm)	Thin (< 5 mm)	
T0 (at removal)	1 2 3	5 5 5	5 5 3	1 / 3
T2 (2 weeks)	1 2 3	5 0 5	4 0 2	0 / 3
T4 (4 weeks)	1 2 3	0 2 2	0 0 0	0 / 5
T6 (6 weeks)	1 2 3	0 1 3	2 1 3	0 / 5

The ELISA test continued to detect PepMV in a number of root pieces 6 weeks after the plants were removed, but successful transmission by sap inoculation was never confirmed (Table 4).

Soil and any remaining fine roots were also sampled at the time test plants were pulled out, and again six weeks later. No transmission of virus was observed when tomato seedlings were grown in soil and fine roots sampled at either occasion. Overall, the risk of carryover of PepMV from one season to the next due to viable virus remaining in roots and soil seems very low.

Following crop removal and chipping, the amount of PepMV that could be detected using the newly validated LAMP assay was investigated. Following positive LAMP results at Nottingham University, compost pieces were also used to inoculate tomato seedlings and *N. benthamiana* plants at Fera, to confirm if the virus detected was viable. The amount of PepMV detected throughout the composting process seemed to drop off quickly after tomato waste

was chipped and left in a stack. At the point of crop removal and chipping in November 2015, all pieces of the waste tested for PepMV returned a positive result, and also resulted in successful infection following sap inoculation of test plants. Tomato waste remained in this stack until 2016, when the formal composting process began. Material in the stack was tested in mid-December 2015, and though PepMV was confirmed by LAMP assay in all the pieces sampled, infection was not achieved following sap inoculation at Fera. Whilst sitting in the stack, the number of sampled compost pieces where PepMV was detected by LAMP assay fell. The formal composting process began in mid-March 2016 (Figure 3), and at this point fresh green leaf from the 2016 crop was incorporated. It is likely that this introduced additional PepMV to the stack, as the number of positive samples increased at this point. Over the course of the composting process, PepMV was detected in a decreasing number of samples as the compost stack was sampled at 10 different points (at 15 and 30 cm deep, five at each depth) weekly. All sap inoculation tests failed to result in infection after the first test using freshly chipped material.

Figure 3. Three rows encompassing the composting of tomato waste at a commercial site. Compost is mixed and put in rows (right), the row is then turned (middle), before finally being left out to dry (left)

Temperatures in the compost stack and rows ranged between 51 to 83°C, with an average temperature of 57.6°C over the 16 weeks sampled. The LAMP assay continued to detect PepMV in sampled compost, but it is likely that though virus particles or fragments remained to be detected, the PepMV did not remain viable for long under composting conditions. In this case, compost would not be re-applied to cropped areas until at least the following year. In conclusion, composting in this way appears to be an effective method for eradication of viable PepMV from chipped tomato waste.

Objective 5 - Validate a method for detection of PepMV in water and test some water samples from UK tomato nurseries

A method for viral particle concentration in water samples, developed at the National Institute of Biology (NIB) Slovenia, was used to concentrate samples collected from three commercial sites. The specific method used cannot be discussed here as it is covered by a confidentiality agreement until the use of the method on PepMV is published. Once the water samples had been collected, they were filtered and concentrated into ten 0.5 ml fractions. These fractions were then diluted and tested in RT-LAMP assays with the CH2 primer.

PepMV was successfully detected in water sampled from two of three commercial sites in 2016 (Table 5).

Table 5. Water samples taken from the irrigation loop on commercial tomato growing sites where PepMV was successfully detected – 2016

Site	Irrigation	Sample	PepMV detected (CH2)
1	Rockwool with pasteurisation	Reservoir (rain and condensed glasshouse water)	✓
		Pre-treatment	✓
		Post-treatment	×
2	Rockwool with pasteurisation	Reservoir (rain)	×
		Pre-treatment	×
		Post-treatment	×
3	NFT (Nutrient Film Technique)	Pre-plants (source + feed)	✓
		Post-plants (drain)	✓

At Site 1, PepMV was detected in a reservoir and pre-disinfection treatment, but not after disinfection treatment (Table 5). The treatment in use was pasteurisation in both cases, and at the time of sampling, equipment was functioning as normal. The reservoir at Site 1 was filled using rainwater and any condensation collected from within the glasshouse, where PepMV was confirmed in the crop. It is likely that this condensate was the virus' point of entry into the reservoir. PepMV was also detected in water sampled from an NFT site, where no disinfection treatment was in place.

Financial Benefits

- % yield loss due to PepMV is difficult to quantify, as it varies by variety and year and will be higher if a severe/necrotic strain or a mixture of strains is present; the disease also causes problems at grading.
- At one commercial site, PepMV was estimated to cost approx. £400,000 for six hectares of tomato cv. Piccolo.
- Availability of a new tool – a LAMP assay for strain-specific detection of PepMV - to increase understanding of the current most important and widespread disease of tomato in the UK.
- Knowledge that, though the CH2 strain remains dominant, EU and US1 strains are also present in UK crops at varying abundance, may help to explain grower observations of the same variety showing more severe symptoms on one nursery than another.
- Once established, the cost of consumables to run a single LAMP assay is around £5. Up to 6 or 12 assays, depending on equipment model, can be run simultaneously.
- Increased confidence that the risk of PepMV carryover in soil is very low.
- Identification of locations where PepMV is persisting on nurseries after crop removal, thereby permitting review and revision of crop-hygiene protocols to address ineffective treatments.

Action Points

- Biosecurity efforts should be maintained even where PepMV is already present, as introduction of additional strains/genotypes appears linked to more severe fruit symptom expression.
- Clean-up year on year appears to be successful, but it is possible that virus can survive in difficult to treat areas of the nursery, such as in electrical equipment and if there is a short turnaround time between crops – only a small amount of virus is required to initiate infection.
- The risk of carryover in soil and roots from one crop to the next appears negligible, though roots left behind represent a greater risk than soil.
- Thorough composting is an effective way to eliminate PepMV from crop debris. Breakdown of viable virus appears to occur relatively quickly, but care should be taken to prevent re-introduction of virus to crops if the compost windrows are located on-site.
- PepMV was detected in irrigation water on two sites, including in source water. This could represent a pathway for spread through crops on the same irrigation loop, and

could possibly reintroduce the virus after clean-up. Disinfection treatment (pasteurisation) successfully eliminated detectable PepMV.

- A review of source waters, and location of disinfection treatments on the water loop may prove beneficial for some growers.

SCIENCE SECTION

Introduction

Pepino mosaic virus (PepMV) was first recorded in in the UK in 1999, causing a variety of symptoms ranging from slight impacts on growth to severe necrosis, with some cultivars more susceptible than others. Control options are limited, with growers altering growing practices to minimise effects on yield. Recently, a mild strain of the virus has been approved in Europe which, through mild strain cross-protection, may offer more effective control from the propagation stage. Currently, five strains of the virus are recognised; though some researchers further split strains to make a total of six. This project focussed on the three strains commonly detected in Europe (CH2, EU and US1). Other recognised strains are US2, LP and PES (Blystad *et al.,* 2015; Davino *et al.*, 2016).

PepMV detection

Detection of PepMV in plants on nurseries is currently usually done by an antibody-based lateral flow detection (LFD) kit which cannot distinguish PepMV strains. Alternatively samples can be sent to Fera for detection by laboratory-based ELISA tests or PCR which are more sensitive but take several days from dispatch to receipt of results. Strain determination is done by sequencing of specific regions of the viral genomes and comparison with sequences on databases.

Loop mediated isothermal amplification (LAMP) assays utilising the Genie machine permit rapid (30 minutes from set-up to result) on-site, very specific and very sensitive assays. The major cost is in selecting primers that accurately detect the specific organism of interest (in this instance, three specific strains of PepMV) and do not cross-react with closely-related organisms (or strains), and devising assay conditions for efficient detection. LAMP assays for detection of the CH2, EU and US1 strains have recently been published (Ling *et al.,* 2013) as cheap alternatives to methods such as reverse transcription-PCR (RT-PCR) or sequencing, which require specialised equipment or sophisticated laboratory conditions. Once established, the cost of consumables to run a single LAMP assay is around £5. Up to 6 or 12 assays, depending on equipment model, can be run simultaneously.

Genie II equipment for conducting LAMP assays is currently available at the University of Nottingham and Fera, for example. ADAS have recently invested in this equipment for use in research projects such as this proposal.

Mild strain cross-protection

Two mild strains have been developed for protecting tomato against infection by severe strains of the same strain(s). A mild strain of a CH2 (isolate 1906) was selected in Belgium and developed by De Ceuster Mestoffen (DCM) and is marketed as PMV-01. This strain has been used in Belgium since 2011 and the Netherlands since 2012. A second mild strain product V10, comprising a mixture of a mild strain of a CH2 strain and a mild strain of an EU strain, has been developed by Valto BV in the Netherlands. Experimental work showed that V10 protected against EU and CH2 strains of PepMV. V10 was reported to have been widely used in commercial crops in the Netherlands since 2014 with good results. When this project started, no mild strain PepMV products were permitted for use in the UK. The mild strain PMV-01 was recently approved (spring 2016) for use in the UK, to be applied to plants on arrival at the commercial nursery.

Testing of PepMV for strain identification was done on a small number of affected crops in the UK in late 2013. The majority of samples were positive for the CH2 strain. Surprisingly, two samples also tested positive for the US1 strain. In order for mild strain cross-protection to be effective, it is necessary for the mild strain inoculant to contain all strains with which the crop may be challenged. Neither of the recently developed mild strain products would provide protection against the US1 strain. Work to develop a mild strain product containing CH2, EU and US1 strains is in progress in the Netherlands and USA.

Symptom severity

Many factors are known to affect symptom severity of PepMV (see: O'Neill, 2014). The occurrence of two different strains in the same plant has been reported to cause severe symptoms in Belgium and the Netherlands. The occurrence of severe PepMV symptoms in some UK crops in 2013 and 2014 may have been due, at least in part, to mixed strain infections (e.g. CH2 + US1).

Survival of PepMV on nurseries

A few nurseries are known to have succeeded in eliminating PepMV after an outbreak, yet on others the disease has occurred many years in succession despite thorough nursery hygiene and disinfection between crops. One potential source of PepMV where it is difficult to implement hygiene and disinfection is the soil. Organic and/or conventional crops grown

in the soil may allow the virus to persist between successive crops in root pieces remaining in the soil after crop removal. In PC 181 we showed that PepMV can occur in tomato roots to at least 30 cm depth in soil. Viable PepMV was detected in roots sampled from the soil 31 days after removal of plants, but not in roots sampled after 57 days. Non-viable PepMV was detected at 57 days. The continuing occurrence of PepMV outbreaks where crops are grown in the soil warrants re-examination of the survival of PepMV in roots in soil.

Tomato crop waste may be composted and then utilised in glasshouses producing tomatoes as a soil mulch or by incorporation. Work on other virus pathogens suggests that effective composting should eliminate PepMV (Noble & Roberts, 2004). There is no published data on the efficacy of aerobic composting on the viability of PepMV or other Potexvirus in tomato crop waste.

Survival of PepMV in water and its detection

Water has been largely overlooked as a virus infection pathway in tomato, most probably because of the low virus particle concentration in water, and the difficulty of detecting viruses at low concentrations. However, there is evidence that PepMV does survive and transmit in water (Mehle *et al*., 2014, Schwarz *et al*., 2010). PepMV (CH2 strain) remained infectious in water at 20 ± 4ºC for up to 3 weeks. It has been shown that the virus (both EU and CH2 strains) can be released from plant roots into the nutrient solutions and can infect healthy plants through their roots, ultimately spreading to green parts where they can be detected after a few months. Irrigation water, especially where it is recycled, is therefore a route that provides significant opportunity for rapid virus spread through a crop. Possibly the sudden occurrence of PepMV-infected plants widely distributed through a crop, as reported by some UK tomato growers, could be the result of spread by recycled irrigation water infested with PepMV. Even if infection of roots and systemic invasion by PepMV into the upper parts of plants occurs only rarely, the virus can be rapidly and effectively spread to neighbouring plants mechanically from just a few widespread plants infected via the roots.

Efficient detection of PepMV in irrigation water requires a method to concentrate the virus particles. Novel methods using filtration before RNA extraction have recently been developed for noroviruses in drinking water (Xu *et al*., 2014) and for *Tomato mosaic virus* in irrigation water (Boben *et al*., 2007); sample sizes of up to 500 ml of water were used. Potentially these methods could be used for PepMV. An alternative method to test water for PepMV is to mechanically inoculate tomato or other indicator plants with the water sample. Although such

a test will indicate viability of the virus, it is slow and resource intensive and is therefore not ideally suited as a method for testing multiple water samples. Also, unlike RT-PCR of a filtered water sample, it is not quantitative.

Materials and methods

Objective 1 – Validate published LAMP assays for rapid detection of CH2, EU and US1 strains of PepMV

Primers, as designed in Ling *et al* (2013) were purchased and used in RT-LAMP assays to test RNA extractions from tomato fruit and leaf samples (Table 6). Each primer type (CH2, EU and US1) was made up into a primer mix containing 152 µl of distilled water, 4 µl of 100 µM B3 primer, 4 µl of 100 µM F3 primer, 20 µl of 100 µM FIP, 20 µl of 100 µM BIP, 20µl of 100 µM LF primer and 20 µl of 100µM LB primer. For the RT-LAMP assay, each reaction mixture consisted of 3.2 µl of distilled water, 3 µl primer mix (as above), 15 µl of Isothermal Mastermix (Optigene, containing a DNA polymerase, optimised reaction buffer, Mg_2SO_4 and dNTPs), 0.5µl of RNAse inhibitor, 1.3 µl of M-MLV reverse transcriptase and 1.5 µl of RNA sample from extraction, giving a total reaction volume of 24.5 µl. The reaction mixtures were then placed in a Genie II (Optigene) platform and heated at 50°C for 10 minutes and 65°C for 30 minutes, followed by a final anneal step ranging from 98-80°C. A positive result would show with an amplification curve on the Genie II touch screen and confirmation of a positive result would be given by the anneal temperature also displayed on the screen.

Table 6. Primer sequences for the RT-LAMP CH2, EU and US1 Ling *et al* (2013) primers

PepMV Strain	Primer Type	Primer Sequence
CH2	F3	5′-CGATGAAGCTGAACAACATTTCC-3′
	FIP	5′-CTTAATGGGTTGATCTTGGTGGAAGCTGTGAGAAAGCTTCACAAAC-3′
	BIP	5′-GGGTTAAGTTTTCCCCAGTTTGAAAATTCCTTCAGTGTTAATCTTGTG-3′
	B3	5′-TCCAGCAATTCCGTGCACAACAA-3′
	Loop F	5′-GGCCTCGCCTTGATGGA-3′
	Loop B	5′-TGGAAAGATCAACTTTGATCAATT-3′
EU	F3	5′-ACCAAGAAGATACAAAATTTGC-3′
	FIP	5′-TRAGACCATCAGCAGGCTGC TGCATTTGACTTCTTCGATG-3′
	BIP	5′-TCAGGCARCCAAATGAGAAAGAAACCTGTGGAGATCTTTTGC-3′
	B3	5′-TGACTTCTCCAAGTGTGG-3′
	Loop F	5′-TGGCAGGGTTGGTGACTC-3
	Loop B	5′-CTAGCTGCTCACTCCGTAGCTAA-3′
US1	F3	5′-GCATTCATACCAAATGGGAG-3′
	FIP	5′-TGCGAACAGCCAAGAAATGT-ATAAATTGCATGAATACCTTACTCC-3′
	BIP	5′-TTGCACAAACTCCACCAAGGACTTAACCCGTCAATGTGTT-3′
	B3	5′-CCATTTCGAACAGGGGAA-3′
	Loop F	5′-TGCTCAGCTTCATCA-3′
	Loop B	5′-TGAAGCCATGAGACTT-3′

The RT-LAMP assay was initially used to test RNA extractions from tomato fruit and leaf samples collected from Sutton Bonington glasshouses. RNA extracts of the EU and US1 strains were obtained from Fera Science Ltd for RT-LAMP testing. It was essential to test samples of all three strains in order to determine if each strain-specific primer was indeed specific and successful at detecting its respective PepMV strain. The primers were then used to test a variety of tomato fruit and leaf samples, swab samples, compost samples and water samples, as discussed in the sections below.

Objective 2 – Determine occurrence of mixed strain PepMV infections in tomato crops

Beginning in 2015, UK commercial sites were sought where crops were showing visible symptoms of *Pepino mosaic virus*. Four crops were assessed on two occasions, approximately one month apart, and two crops were assessed once. Assessments were focused in autumn and spring, where severe symptoms of PepMV are most commonly seen. The crops assessed are summarised in Table 7 below.

Table 7. Detail of the six individual tomato crops visited and assessed over 2015 and 2016 for symptoms of PepMV

Crop	Dates visited	Variety / Scion	Substrate
1	09/09/2015 & 07/10/2015	Piccolo / Maxifort	Organic
2	18/09/2015 & 12/10/2015	Roterno / Maxifort	Rockwool
3	15/10/2015	Piccolo / Maxifort	Rockwool
4	08/03/2016 & 12/04/2016	Piccolo / Maxifort	Organic
5	15/03/2016 & 20/04/2016	Sunstream / Maxifort	Rockwool
6	08/06/2016	Piccolo / Emperador	NFT

In addition, samples were sought exhibiting severe symptoms, such as those produced by necrotic strains of PepMV. Negative samples were also collected from sites reported to be free of the virus.

At each site visit, 30 samples were taken in total. Ten leaf samples were taken from severely symptomatic plants, and 10 from asymptomatic plants, selecting the leaflet three down from the head on each of 10 plants. Additionally, 10 samples of symptomatic tissue, for example chlorotic leaves or marbled/malformed fruit were also taken from the symptomatic plants. Asymptomatic samples were always taken first to avoid cross-contamination with symptomatic samples. Samples were packaged and posted to Nottingham University on the

same day. Here, each individual sample was tested for strains of *Pepino mosaic virus* using the LAMP assay.

As well as taking samples, 100 plants in each crop were selected at random (ten along ten rows) and assessed for symptoms, using a system based on Hanssen *et al*., 2009. Plants were scored for head symptoms such as nettle head, leaf symptoms including chlorosis and necrosis, and stem/petiole symptoms such as excessive twisting or malformation, or streaks appearing on stems. All red trusses were assessed for marbling and flaming on fruit, and for open/malformed fruit. Each symptom was assessed on a 0-3 index, with 0 being no observable symptoms and 3 being awarded where severe symptoms were evident.

Objective 3 – Monitor greenhouse structures and equipment for occurrence of PepMV after crop removal

In autumn 2015 swabs were taken around clean-up on three commercial sites. Swabs were taken as near to crop pull-out as possible, prior to clean-up, from areas where PepMV infection had been confirmed. Swabs were taken with new cotton wool buds, moistened with phosphate buffer. Completed swabs were placed into new sterilin tubes. Fifty swabs were taken at each site, and the locations of swabs and condition of surfaces noted. Glasshouse structures, growing equipment and items such as water coolers, first aid boxes and electrical infrastructure were swabbed. At each site, the clean-up process, including which disinfectant products were used, were noted. A follow up visit occurred at each site as soon after the completion of clean-up as possible, and before the arrival of new plants. The same 50 surfaces were swabbed again. Duplicate swabs were taken on both occasions, with one set sent to Nottingham University, and another sent to Fera Science Ltd on the same day.

On arrival at Nottingham, swabs were tested using the LAMP assay, and following this, a selection of the duplicate swabs at positions which had tested positive for PepMV by LAMP assay were tested for viable virus by sap inoculation of young tomato plants at Fera. On the first swabbing occasion, the LAMP assay and the more traditional ELISA (enzyme-linked immunosorbent assay) test (carried out at Fera) were compared. A fourth site was initially included, but a second visit could not be completed for commercial reasons.

The clean-up processes used at each of the 3 sites sampled are summarised in Table 8 below.

Table 8. Detail of clean-up protocols used on three tomato nurseries - 2015

Site		Clean-up protocol	
	Crop debris	Cleaning	Biocides
Site 1	All removed as far as possible	Glass and irrigation cleaned. Steam cleaning of structure, equipment and staff clothing cleaned	Sanprox P (peroxyacetic acid, hydrogen peroxide & acetic acid) used extensively. Structures wiped with hypochlorite.
Site 2	All removed as far as possible	Glass and irrigation cleaned	Virkon S (peroxygen compounds) on glasshouse structures, floor. Sanprox P on upper structure.
Site 3	Remains in house, covered in plastic	Glass and irrigation cleaned	Mid-structure sprayed with Horticide (glutaraldehyde + quarternary ammonium compounds)

Objective 4 – Determine survival in tomato roots in soil and in composted tomato waste

Roots and soil

This portion of work focussed on an organic crop of tomato cv. Piccolo, grown on the rootstock Maxifort. At the end of the season, plants are cut down and the root system is pulled up out of the soil. The plants selected had infection confirmed by PepMV Lateral Flow Device (LFD) tests. To emulate this process earlier than usual, in order to ensure results were obtained by December 2015, six plants in six different rows were cut down from the crop and the roots pulled up in July 2015, and plants were removed from the area. This was carried out by staff on the host nursery, in order to most effectively replicate the commercial process. Roots remaining in the soil after crop pull-out were sampled at the time the crops were cut down and removed, and at 2 week intervals following this for 6 weeks. Of the six plants cut down, roots from half of the rootzone of three plants were sampled each time to give three replicate samples for each time point. Roots were forked out and five ‘thin’ root pieces (below 5 mm in diameter) and five ‘thick’ root pieces (above 5 mm in diameter if possible) were sampled on each occasion. Wherever possible, samples were taken from different roots rather from different positions along the same root piece. Root pieces were placed in new sterilin tubes and were posted to Fera Science Ltd on the same day for testing by ELISA test.

As this activity would usually occur in autumn, soil temperature at 15 cm depth was recorded at each sampling time so that any different conditions were logged.

An additional test was done to examine the occurrence of soil PepMV in soil and fine root pieces that are inevitably left in soil after crop removal, at transmissible levels. At the first and last sampling occasion, all remaining roots from the rootzone area sampled at that time were collected and placed in a single bag. The weight of these roots was recorded using suitable scales. Approximately 1 Kg of soil was also collected from the three rootzones. Roots and soil were also posted to Fera. On arrival, roots and soil were divided into ten equal portions, mixed with heat sterilised potting soil and used to fill 10 plant pots in which tomato seedlings cv. Moneymaker were then grown on for eight weeks. Plants were observed for development of PepMV symptoms, and were tested for PepMV by ELISA at the end of the eight week period.

Composted tomato waste

A tomato grower practicing on-site composting of tomato waste was visited at the end of the growing season in 2015, when a crop of tomato cv. Piccolo with confirmed PepMV infection was being pulled out and chipped (7th November 2015). Samples were taken of the freshly chipped crop, which was subsequently moved to a stack in the composting shed, where air is blown using fans. Material from this stack was sampled on three subsequent occasions at monthly intervals. A sample of chipped crop was also taken to the ADAS Boxworth pathology laboratory and placed in an incubator at 24°C to act as a control treatment. The full composting process began on 16th March using material from the 2015 chipped crop. The material was mixed with chipped wood and fresh green leaf coming off the newer 2016 crops, and was arranged in windrows. The rows continued to have air blown through, though from this point, use of fans was tightly controlled and the material was covered with a breathable Gore-tex membrane to encourage the composting process. At the seventh sample occasion, the compost entered phase 2 of the process. A sample of the chipped and mixed compost was also transferred to an incubator at ADAS Boxworth and held at 24°C to be sampled as a control. The compost rows were them sampled at weekly intervals. One control sample (T9) failed to arrive, as it was lost in postage. At each sampling occasion, temperature of the stack/row was recorded and photographs of the material were taken. Ten replicate samples were taken each time of both the composting material and the control material. Samples were placed in sterilin tubes and were posted to Nottingham University on the same day for testing by the LAMP assay. Samples were then posted onto Fera so that sap inoculation tests could be carried out with positive samples to check for the presence of viable PepMV.

Mechanical inoculation tests carried out at Fera were performed on tomato cv. Moneymaker and also on *Nicotiana benthamiana* plants. For each sample, one tomato and one *N. benthamiana* were tested. The compost sample was placed in a grinding bag with a spatula full of celite (an abrasive powder) and phosphate grinding buffer. The sample was then ground to a paste using a grinder. The inoculum (compost paste) was then rubbed gently onto 2 to 3 leaves of the selected indicator plants. The leaves were then washed with water. The inoculated plants were kept in a quarantine glasshouse at a temperature between 15 and 18°C for 21 days. The indicator plants were examined for symptoms and then tested by ELISA for PepMV.

Objective 5 - Validate a method for detection of PepMV in water and test some water samples from UK tomato nurseries

Following on from evidence that PepMV may be found in water, water samples were collected from two commercial sites in early 2016. Water was collected from the source (reservoirs), from the drain water after water had passed through the crop (Table 9), and after the disinfection treatment in place at each site (both sites used pasteurisers). A litre of water was sampled at each point on each site, and was posted to Nottingham University the same day. Water samples from both before the rootzone and after were also taken from an NFT crop without a disinfection treatment in place. On arrival at Nottingham University, water samples were filtered and tested for presence of PepMV by the LAMP assay.

Table 9. A summary of water samples taken from commercial tomato sites in 2016 to be tested for the presence of PepMV

Site	Irrigation	Sample
1	Rockwool with pasteurisation	Reservoir (rain and condensed glasshouse water)
		Pre-treatment
		Post-treatment
2	Rockwool with pasteurisation	Reservoir (rain)
		Pre-treatment
		Post-treatment
3	NFT	Pre-plants (source + feed)
		Post-plants (drain)

In 2015, Bethan Warman, an MRes student at Nottingham University, travelled to the National Institute of Biology (NIB) Slovenia, where a method for PepMV concentration in water has been developed. Bethan received training for this concentration technique; however the methodology cannot be discussed here as it is covered by a confidentiality agreement until the work is published.

Results & Discussion

Objective 1 – Validate published LAMP assays for rapid detection of CH2, EU and US1 strains of PepMV

Initial RT-LAMP tests on RNA extracts from Sutton Bonington gave positive results when using the CH2 primer but no positive results were obtained using the EU and US1 primers, indicating that CH2 infection only was present in these samples. In order to validate the EU and US1 primers, EU and US1 PepMV RNA needed to be tested. EU and US1 RNA extracts were obtained from Fera Science Ltd and tested with the RT-LAMP primers. Results revealed that the EU primer was successful at detecting the EU samples and the US1 primers were successful at detecting the US1 samples. However, the EU primers also detected the US1 samples and the US1 primer also gave positive results for the EU samples, as can be seen in Table 10. The CH2 primer detected three of the EU samples and two of the US1 samples. Pure CH2 samples were not tested, as no cross reactivity was present i.e. EU and US1 primers did not pick up CH2 infection.

Table 10. RT-LAMP results for EU and US1 PepMV samples using the Ling et al (2013) EU and US1 primers

	RT-LAMP Amplification time with each primer (mm:ss)		
Sample	CH2	EU	US1
EU 1	20:00	**04:30**	15:30
EU 2	26:45	**04:15**	15:30
EU 3	X	10:45	17:45
EU 4	X	**03:30**	17:30
EU 5	X	**05:30**	15:30
EU 6	25:15	11:30	16:15
US1 1	24:25	10:15	**06:45**
US1 2	27:30	10:30	**05:15**
US1 3	X	11:00	**05:15**
US1 4	X	11:30	**06:15**
US1 5	X	11:45	**06:00**
US1 6	X	14:30	**06:15**

X=no amplification
Bold=fast amplification times (less than 10 minutes), imply a higher initial concentration of this strain.

As can be seen in Table 10, the EU primer amplified the US1 samples at a slower speed than the EU samples (apart from EU 3 and 6) and the US1 primer amplified the EU samples at a slower speed than the US1 samples. There were two potential explanations for why this amplification pattern was seen. Firstly, the primers may have been exhibiting cross-reactivity, meaning that the EU and US1 primers detect both EU and US1 PepMV infection and so are not strain-specific. Alternatively, contaminating RNA may have been present at low levels within the samples, for example the EU samples may have contained low levels of US1 RNA. If this was the case, the US1 primer would detect the EU sample at a slower amplification time, which is what was observed here. EU samples 3 and 6 most likely amplified slower than the other EU samples with the EU primer because samples 3 and 6 may have contained lower viral titres than the other EU samples.

In order to determine if the EU and US1 samples contained contaminating RNA, cloning and sequencing was conducted on two EU and two US1 samples. PCR was conducted on ten transformed colonies for each sample and the products were sent for sequencing. The sequencing results showed that no contaminating RNA was present in the samples. However, if the contaminating RNA was present at a low titre within the samples, it may be that tens or hundreds of transformed colonies needed to be sequenced in order to find the contaminating RNA.

Despite the cloning and sequencing results, it was confirmed that there was no cross-reactivity between primer sets when RNA extractions from tomato leaf and fruit samples collected from site 4 (objective 2) were tested. These samples showed amplification with the CH2 and EU primers, however only one sample gave a positive result when tested with the US1 primer. If there was cross-reactivity between the EU and US1 primers, all samples that amplified with the EU primer would have also amplified with the US1 primer.

It can also be seen that the CH2 primer detected three of the EU samples and two of the US1 samples. The amplification times for these EU and US1 samples were slow, with the fastest of the detected samples amplifying after 20 minutes with the CH2 primer. The fact that not all of the EU and US1 samples were detected by the CH2 primer, and the fact that those detected were amplified slowly, suggests that samples EU 1, 2 and 6 and US1 1 and 2 contained low levels of contaminating CH2 RNA, resulting in their amplification when using the CH2 primer.

In conclusion, the Ling et al (2013) primers were strain- specific and were successful at detecting PepMV infection. The RT-LAMP assay using these primers was rapid, with the majority of PepMV-infected samples tested (as part of objective 2, below) showing as a positive result within 10 minutes when using the CH2 primer. The EU primer was able to amplify EU samples quickly, with EU sample 4 from Fera Science Ltd showing as a positive

results after 3 minutes and 30 seconds. The US1 primer also showed rapid amplification when used on the Fera Ltd US1 samples, with the fastest amplification time observed with these samples being 5 minutes and 15 seconds.

The annealing temperature can be used as a method of validating which PepMV strain is present within a sample. The CH2, EU and US1 primers all gave different annealing temperatures with a positive result, as can be seen in Table 11 below. The CH2 primer gave the lowest anneal temperature of approximately 85°C, followed by the US1 primer with a temperature of approximately 86°C and the highest anneal temperature was given by the EU primer at over 87°C. If a universal primer that detected all three of these PepMV strains was used, the anneal temperature could be used to determine which strain was present within the sample. However the issue with using a universal primer is that it would not be possible to determine if mixed-strain infection was present in the sample as only one anneal temperature would be displayed.

Table 11. Anneal temperatures given by the different strain-specific primers when tested on 6 samples from site 3.

	Anneal temperatures (°C) given from each primer		
Sample	CH2	EU	US1
1	85.00	87.34	86.10
2	84.84	87.43	86.09
3	84.94	87.52	86.24
4	84.85	87.48	86.10
5	84.79	87.47	86.13
6	84.77	87.30	85.96

The amplification time can be used to provide quantification of viral RNA concentration within a sample. A sample with a known viral RNA concentration can be used to create a standard curve. Serial dilutions of the sample can be made to see how reductions in the viral concentration affect the speed of amplification. However, due to the experimental design used in this study, a standard was not used and so only a rough correlation can be implied between amplification time and viral RNA concentration, rather than specific quantification.

The RT-LAMP method may be favoured over the use of ELISA to test for the presence of PepMV infection. The main advantages of RT-LAMP are the speed at which a positive result can be confirmed, tests being strain specific, and the higher sensitivity exhibited by this technique over ELISA. One other significant advantage of using RT-LAMP over ELISA is that portable heating instruments, such as Genie II from Optigene, could allow the LAMP method to be conducted on site, giving rapid confirmation of PepMV infection and eliminating the need for samples to be sent for laboratory testing.

Objective 2 – Determine occurrence of mixed strain PepMV infections in tomato crops

The specific symptoms observed in the crops assessed varied from site to site and with sample time (spring vs. autumn), though the symptoms seen in Piccolo did appear broadly similar across sites and season. The crops assessed differed in the proportion of fruit to show symptoms, with fruit symptoms being relatively common in some crops, but near absent in others. All severe symptoms in fruit were recorded at assessments later in the year. Different varieties close to one another on the same site also differed in their symptom expression, though presumably they had the same or similar mixture of PepMV strains present. Severe symptoms of the stem and petiole were recorded rarely, with necrotic spotting observed on stems or petioles on only one occasion, on a single plant (Site 2, April 2016). The incidence and severity of typical PepMV symptoms are summarised in Table 12 below. Example photographs of the different symptom types are shown in the Grower Summary.

Table 12. Detail of the six individual tomato crops visited and assessed over 2015 and 2016 for symptoms of PepMV, and the symptoms typically observed.

Crop	Dates visited	Variety / Scion	Substrate	Main symptoms observed
1	09/09/2015 & 07/10/2015	Piccolo / Maxifort	Organic	Chlorotic spotting, some marginal necrosis
2	18/09/2015 & 12/10/2015	Roterno / Maxifort	Rockwool	Chlorotic spotting, fruit marbling, malformation
3	15/10/2015	Piccolo / Maxifort	Rockwool	Severe chlorosis, some malformed fruit, nettle head
4	08/03/2016 & 12/04/2016	Piccolo / Maxifort	Organic	Nettle head, chlorotic spotting, slight necrosis
5	15/03/2016 & 20/04/2016	Sunstream / Maxifort	Rockwool	None initially; slight necrosis and distortion
6	08/06/2016	Piccolo / Emperador	NFT	Slight necrosis & chlorosis, Nettle head, some fruit marbling

Assessments of likely symptoms of PepMV are summarised in Tables 13-18. Tables 17 and 18 also compare the strains detected with the instances of especially severe symptoms.

Table 13. Occurrence of PepMV foliar symptoms in six tomato crops – autumn 2015 and spring 2016 (100 plants examined in each crop)

Crop	Site	Variety	Visit	Date	Incidence of foliar symptoms of PepMV (% plants affected)				
					Chlorotic spots	Necrotic spots/margins	Petiole symptoms (distortion)	Stem symptoms (distortion)	Head symptoms (nettle head)
1	Site 1	Piccolo	1	9 9 15	26	55	14	9	30
			2	7 10 15	17	47	25	0	0
2	Site 2	Roterno	1	18 9 15	54	0	7	3	6
			2	12 10 15	46	1	10	0	10
3	Site 1	Piccolo	1	8 3 16	67	1	32	9	64
			2	12 4 16	25	65	13	0	49
4	Site 2	Sunstream	1	15 3 16	7	0	0	0	4
			2	20 4 16	10	8	30	4	33
5	Site 3	Piccolo	1	19 10 15	40	24	54	17	3
6	Site 4	Piccolo	1	08 06 16	17	60	39	6	34

Table 14. Occurrence of PepMV fruit symptoms in six tomato crops – autumn 2015 and spring 2016 (100 plants examined in each crop)

Crop	Site	Variety	Visit	Date	Incidence of fruit symptoms of PepMV (% plants affected)			
					Marbling	Flaming	Open fruit	Proportion of trusses affected
1	Site 1	Piccolo	1	9 9 15	3	0	0	1.4
			2	7 10 15	1	0	0	0.5
2	Site 2	Roterno	1	18 9 15	28	19	3	21.3
			2	12 10 15	25	22	4	42.0
3	Site 1	Piccolo	1	8 3 16	0	0	0	0
			2	12 4 16	11	11	1	11.6
4	Site 2	Sunstream	1	15 3 16	0	0	3*	1.7*
			2	20 4 16	3	5	5	5.7
5	Site 3**	Piccolo	1	19 10 15	54	3	3	39.3
6	Site 4	Piccolo	1	08 06 16	17	9	3	11.7
	*possibly due to insect damage **second assessment not possible							

Table 15. Severity of PepMV foliar symptoms in six tomato crops – autumn 2015 and spring 2016

Crop	Site	Variety	Visit	Date	Mean severity of foliar symptoms of PepMV (0-3 index) for all 100 plants				
					Chlorotic spots	Necrotic spots/margins	Petiole symptoms (distortion)	Stem symptoms (distortion)	Head symptoms (nettle head)
1	Site 1	Piccolo	1	9 9 15	1.1	1.1	1	1	1
			2	7 10 15	1.1	1.1	1.1	0	0
2	Site 2	Roterno	1	18 9 15	1.1	0	1	1	1
			2	12 10 15	1.1	1	1	0	1.8
3	Site 1	Piccolo	1	8 3 16	1.4	1	1.7	1	1.9
			2	12 4 16	1.1	1.4	1.4	0	1.4
4	Site 2	Sunstream	1	15 3 16	1	0	1.4	0	1
			2	20 4 16	1	1	1.3	1.5	1.2
5	Site 3	Piccolo	1	19 10 15	1.7	1.1	1.1	1	1.5
6	Site 4	Piccolo	1	08 06 16	1.2	1.4	1.5	1.5	1.6

Table 16. Severity of PepMV fruit symptoms in six tomato crops – autumn 2015 and spring 2016

Crop	Site	Variety	Visit	Date	Mean severity of fruit symptoms of PepMV (0-3 index) for all 100 plants			
					Marbling	Flaming	Open fruit	Proportion of trusses affected
1	Site 1	Piccolo	1	9 9 15	1	0	0	1.4
			2	7 10 15	1	0	0	0.5
2	Site 2	Roterno	1	18 9 15	1.1	1.2	2	21.3
			2	12 10 15	1.3	1.3	1.8	42.0
3	Site 1	Piccolo	1	8 3 16	0	0	0	0
			2	12 4 16	1	1	1	11.6
4	Site 2	Sunstream	1	15 3 16	0	0	1	1.7
			2	20 4 16	1	1	1	5.7
5	Site 3	Piccolo	1	19 10 15	1.8	1	1.7	39.3
6	Site 4	Piccolo	1	08 06 16	1.2	1	1	11.7

Table 17. Occurrence of severe foliar symptoms of PepMV in six tomato crops– autumn 2015 and spring 2016

Crop	Site	Variety	Visit	Date	Proportion of all plants with an index ≥ 2 (%)					PepMV strains detected		
					Chlorotic spots	Necrotic spots/margins	Petiole symptoms (distortion)	Stem symptoms (distortion)	Head symptoms (nettle head)	CH2	EU	US1
1	Site 1	Piccolo	1	9 9 15	2	5	0	0	0	✓	-	-
			2	7 10 15	2	5	2	0	0	✓	-	-
2	Site 2	Roterno	1	18 9 15	4	0	0	0	0	✓	-	✓
			2	12 10 15	0	0	0	0	7	✓	-	✓
3	Site 1	Piccolo	1	8 3 16	20	0	14	0	37	✓	-	-
			2	12 4 16	3	21	4	0	18	✓	-	-
4	Site 2	Sunstream	1	15 3 16	0	0	2	0	0	✓	-	-
			2	20 4 16	0	0	8	2	4	✓	-	-
5	Site 3	Piccolo	1	19 10 15	18	2	1	0	8	✓	✓	✓
6	Site 4	Piccolo	1	08 06 16	3	22	15	2	13	✓	✓	✓

Table 18. Occurrence of severe fruit symptoms of PepMV in six tomato crops– autumn 2015 and spring 2016

Crop	Site	Variety	Visit	Date	Proportion of all plants with an index ≥ 2 (%)			Proportion of trusses with an index ≥2	PepMV strains detected		
					Marbling	Flaming	Open fruit		CH2	EU	US1
1	Site 1	Piccolo	1	9 9 15	0	0	0	0	✓	-	-
			2	7 10 15	0	0	0	0	✓	-	-
2	Site 2	Roterno	1	18 9 15	3	3	2	6	✓	-	✓
			2	12 10 15	7	5	3	9	✓	-	✓
3	Site 1	Piccolo	1	8 3 16	0	0	0	0	✓	-	-
			2	12 4 16	0	0	0	0	✓	-	-
4	Site 2	Sunstream	1	15 3 16	0	0	0	0	✓	-	-
			2	20 4 16	0	0	0	0	✓	-	-
5	Site 3	Piccolo	1	19 10 15	19	0	1	19	✓	✓	✓
6	Site 4	Piccolo	1	08 06 16	3	0	0	9	✓	✓	✓

When tested by the LAMP assay, the majority of samples returned positive results for CH2 PepMV. At Site 1, for all crops tested, CH2 was the only strain detected. Both CH2 and US1 was detected at Site 2 and Site 3 in 2015, where some of the most severe symptoms were also observed. The US1 strain was only detected in one of the samples on Site 4. The EU strain was detected at Sites 3 (sampled in 2015) and 4 (sampled in 2016), meaning all three strains tested for were detected on these sites. Table 18 identifies these two sites as those with a greater proportion of trusses expressing severe fruit symptoms. Whether the plants sampled were obviously symptomatic or not, and what type of tissue was sampled, did not seem to have great bearing on the strains detected, though in crops with severe symptoms, it was often difficult to find plants that were entirely asymptomatic. A summary of results from the LAMP assay from each site are summarised in Table 19 below.

Table 19. PepMV strains confirmed by LAMP assay (% of samples tested) at four tomato nurseries – 2015 and 2016

Site	Cultivar	Date	Symptomatic plants			Symptomatic tissues			Asymptomatic plants		
			CH2	EU	US1	CH2	EU	US1	CH2	EU	US1
1	Piccolo	09 09 15	100	0	0	100	0	0	100	0	0
		07 10 15	100	0	0	100	0	0	100	0	0
	Piccolo	08 03 16	100	0	0	100	0	0	100	0	0
		15 03 16	100	0	0	100	0	0	100	0	0
2	Roterno	18 09 15	100	0	10	100	0	0	100	0	0
		12 10 15	100	0	0	100	0	0	100	0	10
	Sunstream	15 03 16	56	0	0	50	0	0	58	0	0
		20 04 16	0	0	0	10	0	0	20	0	0
3	Piccolo	19 10 15	100	100	100	100	100	80	100	100	100
4	Piccolo	08 06 16	100	100	0	0	100	10	100	100	0

In addition to the sites visited and sampled, crop samples of severe symptoms were sought. Both severe samples taken also came from Site 1, though from a different glasshouse on the site, quite far away from the monitored area (Table 20). These samples also tested positively for only the CH2 strain, with the leaf sample being a likely necrotic strain. Additional samples were also received from other commercial sites with very few to no obvious symptoms, but the CH2 strain was confirmed. This confirms the variability of UK populations of PepMV, and the variability of symptoms observed. Severe samples were also tested for presence of *Tomato spotted wilt virus*, which was not detected.

Table 20. A summary of PepMV strains detected by LAMP assay in samples received showing severe symptoms

Sample	Site	Date	Variety	Sampled material	Results of LAMP assay:		
					CH2	EU	US1
1	1	04 04 16	Lyterno	Necrotic leaves	100	0	0
2	1	31 05 16	Brioso	Marbled fruit	100	0	0

Every sample taken at Site 1, in both years, tested positive for PepMV. Site 1 also had plants showing relatively severe symptoms, especially for the symptom termed 'nettle-head'. However, the main crops monitored at Site 1 had no severe fruit symptoms. It is possible that the crops became infected on this site earlier, and thus the plants had more of a chance to grow through symptoms before trusses were set. Additionally, it is known that growing conditions and different crop steering methods influence symptom expression on a site by site basis (Hanssen *et al.,* 2009). Growing temperature is also known to have a strong effect on symptom expression (Hasiow-Jaroszewska *et al*., 2015). One of the additional severe samples taken from another area of Site 1 did show severe fruit symptoms, and again only the CH2 strain was detected by LAMP assay. It may be that this area of the site was infected later in the year than the area more closely monitored, and so affected the ripening of fruit on the trusses. Additionally, strains of PepMV are known to have an extremely high mutation rate, with minimal genetic changes distinguishing mild, necrotic and yellowing strains (Hasiow-Jaroszewska *et al*., 2015; 2013). It is possible that such mutations occur and become dominant in tomato crops part way through the season, with the arrival of more severe symptoms.

Site 2 had minimal foliar symptoms in 2015, but some of the most severe fruit symptoms recorded. However, in 2016, at the time of Visit 1 (15 March), symptoms of PepMV had not yet been seen on site. Around half of samples taken, however, did test positive. At both visits in 2016 possible symptoms of PepMV were minimal. Different varieties were assessed year on year at Site 2, and it could be that these differences are due to their different responses to PepMV. When this site was sampled late in 2015, as well as CH2, US1 was also detected at low levels, whereas in early 2016, CH2 only was detected. It is possible that the presence of a mixed infection is what caused symptoms to be more severe in autumn 2015, and multiple strains had not yet been allowed to infect the new crop early in the 2016 season. It was notable that the US1 strain was detected at a low incidence at this site, and yet was ubiquitous at Site 3. On revisiting Site 2 later in 2016, more severe symptoms of PepMV had been observed, but overall it was noted that symptoms were milder than in previous years.

Site 3 had the most severe symptoms, and all 3 strains of PepMV were detected in almost every sample. All three strains were also detected at Site 4. Symptoms were not as severe as at Site 3 overall, though both fruit symptoms, and foliar symptoms such as nettle-heading were observed in Piccolo at Site 4. US1 was detected at a much lower frequency at Site 4 than at Site 3 (where it was detected in 100% of samples), and it could be the increased incidence of the US1 strain that results in more widespread, severe symptoms. It is unclear what influenced the relative frequencies of each strain in the crops where mixed infections were found, but could be related to growing conditions, or possibly the order in which they are introduced to the crop.

Sites on which only the CH2 strain was detected at high incidence early in the season typically had foliar symptoms with very few severe fruit symptoms. At sites where both CH2 and EU (and also US1 in the case of Site 3), fruit symptoms such as marbling were more often seen. Though previously the dominant strain of PepMV in Europe, the EU strain is now most commonly found with CH2 in mixed infections (Ling *et al.,* 2013), as was the case here. It also appears that tomato cv. Piccolo is less likely to develop classic fruit marbling symptoms of *Pepino mosaic virus*, as the most severe marbling was observed on larger vine varieties (cvs Roterno & Brioso), and only reached notable levels on Piccolo at Site 3, where all three strains were detected.

Objective 3 – Monitor greenhouse structures and equipment for occurrence of PepMV after crop removal

When LAMP and the standard ELISA test were compared, the results largely agreed (Table 21). However, the LAMP assay reported some positive results where the ELISA was negative. The LAMP assay may be more sensitive, or it is possible that the LAMP assay has a greater propensity to detect unviable virus or fragments of PepMV RNA. One possible explanation for this may be due to proteins breaking down more quickly in the environment than the target RNA. The lower sensitivity of the ELISA method to low concentrations of virus when compared to LAMP testing has also been observed in Davino *et al.,* 2016.

Table 21. Detection of PepMV in swab samples from glasshouses on 3 nurseries before and after end of season clean-up and disinfection

Site	Incidence of positive tests (%)			
	Pre-clean up		Post-clean up	
	LAMP	ELISA	LAMP	Sap inoculation
1	76	30	20	0
2	86	26	44	0
3	98	-	68	0

- Test not carried out

After the clean-up process at all sites there was a drop in the number of positive swabs (Table 22). The surfaces where detectable PepMV was most likely to remain were those in close, sustained contact with plant material (Tables 22 & 23). Additionally, electrical switches, circuit boards and equipment pose an issue as these surfaces are difficult to disinfect thoroughly and safely. A full list of locations that tested positive for PepMV before and after clean-up can be found in Appendix 1.

Table 22. Summary of locations positive for PepMV by LAMP assay after end of season disinfection at three sites - 2015

	No. of positive LAMP tests		
	Electricals	Close contact with crop/leaf/fruit	Other
Site 1	2	4	4
Site 2	4	14	4
Site 3	5	18	8

Table 23. Details of locations positive for PepMV by LAMP assay after end of season disinfection at three sites - 2015

	Locations of positive LAMP tests		
	Electricals	Close contact with plant material	Other
Site 1	circuit board, trolley of electricals	bracket, 3 x trolley	First aid box, pipe on wall 2 x door handle
Site 2	Switch, 3 x electrical panel	2 x heating pipe, 4 x trolleys, 3 x green waste cage, 2 x bracket, stanchion, picking crate, mypex	spray boom, 3 x concrete path
Site 3	moth light, 2 x charge point, 2 x switch	row label, mypex, chain, 2 x stanchion, bracket, 2 x picking crate, metal support, grow pipe, green waste bin, 2 x trolleys, heating pipe, 2 x drip pegs, 2 x drip lines	whiteboard, 2 x door switch, 2 x forkilift, 2 x hand sanitiser, door handle

Following testing by the LAMP assay, a sub-sample of positive swabs were tested by sap-inoculation tests at Fera, to determine if the PepMV detected was still viable and capable of causing disease. None of the swabs taken at any point produced tomato plants that tested positive for PepMV by ELISA. Seemingly, the majority of PepMV remnants fail to be viable after disinfection, though the virus may still be detected. However, only a small amount of viable PepMV needs to remain after end of year clean up to initiate an infection in the new crop, so every care should be taken. In addition, effective clean up practices also eliminate carryover of other viruses, and fungal and bacterial disease agents.

Objective 4 – Determine survival in tomato roots in soil and in composted tomato waste

Roots

Over the course of sampling, a trend for a reduction in detectable PepMV was observed when tested by ELISA (Table 24). Results also showed that PepMV was recovered more often from thick roots than thin roots, though at T6 this trend was not conserved. By the final sampling date, 6 weeks after plants had been pulled out, finding roots of appropriate size proved difficult, and the roots sent for sampling at this point were relatively small.

Table 24. Detection of PepMV by ELISA and detection of viable PepMV by transmission test in root pieces sampled from soil at intervals (0-6 weeks) after plant removal

Sample time	Rep	No. roots positive (of 5)		No. positive roots shown to have viable PepMV
		Thick (≥5 mm)	Thin (< 5 mm)	
T0 (at removal)	A B C	5 5 5	5 5 3	1 / 3
T2 (2 weeks)	A B C	5 0 5	4 0 2	0 / 3
T4 (4 weeks)	D E F	0 2 2	0 0 0	0 / 5
T6 (6 weeks)	D E F	0 1 3	2 1 3	0 / 5

Soil temperature at 15 cm depth was also recorded at each sampling, in order to compare conditions in mid-summer (when sampling occurred) to conditions in autumn/winter, when the crop would be pulled out and replaced by commercial growers, which may be closer to 18 °C (Table 25). Evidently, the temperatures recorded in summer are slightly warmer than those usually recorded in winter (usually around 18-21°C) around the usual time of crop pull-out by commercial organic growers. This could mean an increased rate of degradation of PepMV was observed than may be achievable in winter.

Table 25. Soil temperature recorded at 15 cm depth after plant removal and subsequent sampling (Objective 3) - 2015

Sample time	Date	Soil temperature (°C)
T0	29 06 15	23.3
T2	13 07 15	26.3
T4	27 07 15	27.1
T6	10 08 15	24.7

However, as successful transmission to tomato seedlings was not confirmed past T0, it is likely that the time between organic crops is sufficient for any viable PepMV in the soil to degrade. Therefore, it is unlikely that soil and root fragments in organically cropped areas represents a significant source of infective virus for the next year's crops.

Soil

For the soil sampled at both T0 and T6, no transmission was confirmed when tomato seedlings were grown in the soil for the test period of 8 weeks. This result indicates that any PepMV remaining in soil or very fine root pieces after crop removal was insufficient to result in transmission to the test plants.

Although reassuring, one cannot conclude, however, that this would necessarily always be the case; a positive transmission test might occur if a greater volume of nursery soil was used in each test pot, or the plants were grown for a longer period of time (i.e. probability of detecting a very low occurrence in the soil increases with test volume and duration).

Composted tomato waste

In autumn 2015, a site was visited at crop pull-out where tomato waste was composted on site (Table 26). A sample of crop, cv. Piccolo, was taken immediately after it had been chipped. This sample, T0, was tested for presence by the ELISA method, and all ten replicates tested were reported as positive for PepMV. Additionally, when tested with the sap inoculation method, all inoculated plants subsequently tested positively for PepMV.

Table 26. A summary of average stack temperatures (taken at 15-30 cm deep into the stack, average of 10 measurements) and timing of key events over the course of the composting process

Sample	Date	Composting stage	Average temperature (°C)
T1	18 12 15	Stack – awaiting incorporation into rows	68.0
T2	26 01 16	Stack – awaiting incorporation into rows	65.5
T3	23 02 16	Stack – awaiting incorporation into rows	64.3
T4	16 03 16	Incorporated into rows with fresh green leaf	60.3
T5	23 03 16	Compost row	83.6
T6	30 03 16	Compost row	72.5
T7	05 04 16	Compost row has been turned	54.0
T8	12 04 16	Compost row	51.0
T9	26 04 16	Composting completed, entered final drying phase	53.1

A high temperature (51-68°C) was recorded in the composting heap at all sample times (Table 26). *Tobacco mosaic virus* (TMV) requires a peak compost temperature in excess of 68ºC and a composting period longer than 20 days for eradication. However, TMV is degraded in compost over time, and can be eradicated after a composting period of 26 weeks even at a low temperature (31ºC) (Noble & Roberts*,* 2004). Based upon what is known about degradation of TMV, and the results of sample testing for PepMV in this project, temperatures in the compost stack appear to be sufficient for elimination of PepMV.

Figure 4 illustrates the difference between chipped tomato waste part way through the composting process (at T4, approx. 4 months after chipping) and freshly chipped tomato that was held in an incubator at a steady 24°C (control at T4). The control chippings were extremely wet, and foul smelling, with evidence of bacterial rot with a small amount of fungal growth. This is in comparison to the composting waste, which was dry without an unpleasant odour and devoid of any visible fungal or bacterial growth.

Figure 4. Compost taken from the compost row after construction at T4 (left) in comparison to the control treatment, where freshly chipped tomato waste was held at a steady 24°C for 4 months (right)

Results of PepMV testing, on compost sampled at intervals by LAMP, sap inoculation and subsequent ELISA are summarised in Tables 27 and 28 below. The compost stack evidently achieved more favourable conditions for activity of composting microbes and for breakdown of PepMV than the constant temperature control with PepMV consistently detected in more control samples that in the compost.

Table 27. Detection of PepMV in composting tomato waste (compost) and in crop waste held in the dark at a constant temperature of 24°C (control) by three test methods at intervals from November 2015 to April 2016

		Percentage of positive samples using each test		
Sample time (date tested)	Sample type	LAMP assay	Sap inoculation	ELISA after sap inoculation
T0 (07/11/15)	Compost	X	100	100
	Control	X	100	100
T1 (18/12/15)	Compost	100	0	0
	Control	100	0	0
T2 (26/01/16)	Compost	100	0	0
	Control	100	0	0
T3 (23/02/16)	Compost	70	0	0
	Control	100	0	0
T4* (16/03/16)	Compost	100	0	0
	Control	X	0	0
T5 (23/03/16)	Compost	80	0	0
	Control	100	0	0
T6 (30/03/16)	Compost	30	0	0
	Control	90	0	0
T7 (05/04/16)	Compost	70	0	0
	Control	100	0	0
T8 (12/04/16)	Compost	70	0	0
	Control	100	0	0
T9 (26/04/16)	Compost	10	X	X
	Control	X	X	X

* = Samples incorporated into windrows with other plant material

X = Samples not received/not tested

Sap inoculations did not result in observable symptoms of PepMV at any point in the composting process, following T0, where freshly chipped pieces of infected tomato were successful in causing infection. This was confirmed by ELISA test on the plants sap inoculated with T0 samples, but never subsequently in the process. These results suggest that though break down of PepMV was more efficient in the compost than in the control, even PepMV in the control sample (24 °C) failed to be viable after a relatively short time (6 weeks).

Following the initial tests at T0, the number of samples testing positive for PepMV gradually reduced until T4. At this point, all samples again tested positive for PepMV by LAMP assay. Introducing fresh green leaf from 2016 crops (which were sampled as part of Objective 2, with PepMV infection confirmed) at this point introduced new PepMV particles to the compost mix, though it was not found to be capable of causing new infections. By the end of sampling, even the more sensitive LAMP test was returning very few positives, and it can be concluded that thorough composting is an effective method of disposing of tomato waste infected by PepMV.

Table 28. Comparison of LAMP assay amplification time in compost and constant temperature control samples at intervals from November 2015; times approaching 30 minute amplification times were taken as Not Detected (ND)

Sample time (x weeks from T0)	Average amplification time (minutes)	
	Control	Compost
T1 (6 week from T0)	10:36	12:48
T2 (12 weeks from T0)	12:25	18:33
T3 (16 weeks from T0)	11:28	18:54
T4* (19 weeks from T0)	X	15:42
T5 (20 weeks from T0)	13:57	16:32
T6 (21 weeks from T0)	13:33	17:15
T7 (22 weeks from T0)	14:27	18:51
T8 (23 weeks from T0)	14:17	17:51
T9** (25 weeks from T0)	X	23:00

*control replaced with new compost mix, same as compost
**control sample lost in postage

The control samples also always resulted in a quicker amplification time than the compost samples (Table 28). A higher amplification time indicates a lower initial PepMV concentration, and vice versa, indicating a slower degradation of PepMV in the control. A trend for increasing amplification time can be seen throughout the course of the trial, indicating a drop in concentration of PepMV from the time the tomato crop was chipped. However, no standard curve was used in this experimental design and so, although a general trend can be implied between the viral concentration and the amplification time, the amplification time cannot provide specific quantification of RNA concentration within the samples.

Objective 5 - Validate a method for detection of PepMV in water and test some water samples from UK tomato nurseries

Water was sampled from two sites with recirculating irrigation systems in spring 2016. Table 29 shows where PepMV was detected in water samples.

Table 29. Samples taken from the irrigation loop on commercial tomato growing sites where PepMV was successfully detected – 2016.

Site	Irrigation	Sample	PepMV detected (CH2)
1	Rockwool with pasteurisation	Reservoir (rain + glasshouse condensate)	✓
		Pre-treatment	✓
		Post-treatment	×
2	Rockwool with pasteurisation	Reservoir (rain)	×
		Pre-treatment	×
		Post-treatment	×
3	NFT	Pre-plants (source + feed)	✓
		Post-plants (drain)	✓

The method developed for concentrating water samples to enable subsequent testing for the presence of PepMV was successful. PepMV was detected in the water sampled from two of the three sites visited. It should be noted that Site 2 was also tested as part of Objective 2, and had relatively low levels of PepMV detected in the crops sampled at the time of sampling,

which may explain the absence of the virus in pre-treatment water compared to presence at the other two sites. Sites 1 and 3 had infection confirmed extensively in crops sampled as part of Objective 2 at the time water was tested.

PepMV was detected in both the reservoir and pre-disinfection treatment water sampled at Site 1, but was not present in post-disinfection treatment water. This implies that the disinfection treatment present (a pasteuriser) effectively removed virus from the water, preventing it being recirculated. At Site 1, PepMV was also detected in the reservoir. Sources of water for this reservoir are rain water and also water condensing on glasshouse structures, and therefore likely to be contaminated with PepMV. It is also possible that plant debris may be entering the rain water collection system. On Site 3, a glasshouse utilising Nutrient Film Technique, PepMV was detected in both the irrigation water going into gutters, and in the drain. This is not surprising, as no disinfection treatment is present and the crops in the area had confirmed PepMV infection.

As irrigation water may represent a fast and efficient way to facilitate spread of viruses such as PepMV (Mehle *et al*., 2014), growers should be aware of the risk of transmission between separate glasshouse areas (where mechanical transmission can potentially be avoided) served by the same irrigation loop.

Conclusions

Conclusions from this project are broken down by objective.

Objective 1 – Validate published LAMP assays for rapid detection of CH2, EU and US1 strains of PepMV

- The Ling et al (2013) primers were effective as detecting strain-specific PepMV infection.
- Amplification of positive samples was rapid: the fastest amplification times seen were approximately 6 minutes, 3 minutes and 5 minutes when using the CH2, EU and US1 primer, respectively.
- Different anneal temperatures of approximately 85°C, 86°C and 87°C were given when using the CH2, US1 and EU primers, respectively. Therefore the anneal temperature could be used to confirm a positive result for a particular strain.

Objective 2 – Determine occurrence of mixed strain PepMV infections in tomato crops

- The majority of UK infections monitored appear to be due to the CH2 strain of PepMV, known to be prevalent in Europe currently.

- Overall, mixed infections, especially those including the US1 strain, resulted in more severe symptoms on fruit.
- As observed in commercial crops, it appears that early infection reduces subsequent severe symptoms on developing fruit.
- Severe fruit symptoms appear more common in large vine varieties than on the variety Piccolo, where fruit symptoms are more likely if a mixed infection is present.

Objective 3 – Monitor greenhouse structures and equipment for occurrence of PepMV after crop removal

- The LAMP assay appears more sensitive than conventional ELISA for PepMV.
- All sap inoculation tests were negative after clean-up, indicating that in general best practice clean-up methods are effective.
- At Site 3, where the least extensive clean-up protocol was followed, the LAMP test gave more positive results, though sap inoculations were all still negative.
- Anecdotally, many sites find it difficult to prevent re-infection, so it is possible some viable PepMV persists from year to year.

Objective 4 – Determine survival in tomato roots, in soil and in composted tomato waste

Roots

- PepMV continued to be detected in tomato roots left in glasshouse soil up to 6 weeks after crop removal (the usual interval between pull-out and re-planting).
- After the first sampling at time of pull out, transmission of PepMV to tomato seedlings was not confirmed.
- It is possible that non-viable virus was detected by ELISA test, and as no transmission was confirmed it is likely that 6 weeks is sufficient to prevent carry over.

Soil

- No transmission of virus was observed when tomato seedlings were grown in soil and fine roots sampled at crop pull-out, and sampled again six weeks later.

Compost

- Though the LAMP assay continued to detect PepMV throughout the composting process, subsequent sap inoculation using sampled compost failed to produce infection in tomato seedlings. Presumably, though virus particles or fragments

remained in the compost sampled for the LAMP assay to detect, the PepMV did not remain viable for long under composting conditions.

Objective 5 - Validate a method for detection of PepMV in water and test some water samples from UK tomato nurseries

- It was possible to detect PepMV strain CH2 in sampled water by LAMP assay after the samples had been filtered and concentrated using a novel method developed at the National Institute of Biology (NIB) Slovenia.
- PepMV was detected in a reservoir and pre-disinfection treatment, but not after disinfection treatment (pasteurisation).
- Pasteurisation appeared effective in removing PepMV from recycled irrigation water.
- The water source tested positive at one site; a pathway was identified by which some water from the glasshouse could enter the reservoir.
- PepMV was also detected in applied and drainage water sampled from an NFT site, where no disinfection treatment was in place.

Knowledge and Technology Transfer

Article in AHDB Grower, Utilising a LAMP assay to advance understanding of Pepino mosaic virus, Publication planned for November 2016.

Powerpoint presentation at an appropriate event, TBC

AHDB Factsheet, *Pepino mosaic virus* of tomato – new results on strains, symptoms and persistence. In preparation.

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Appendices

Appendix 1. All on site locations swabbed before and after end of year clean-up and disinfection, and results of subsequent testing (Objective 3)

Site 1

	Result of LAMP assay	
Location swabbed	Pre-clean	Post-clean
Concrete at row 39	✓	×
Water cooler	×	×
Concrete at row 34	✓	×
Trolley number 364	✓	×
Ladder rungs trolley 362	✓	✓
Inside door handle	✓	✓
Inside main circuit board box at row 41	×	✓
Small switch row 39	✓	×
Glass at end row 43	×	×
Glass at end row 46	×	×
Heating pipe row 33	✓	×
Heating pipe row 46	✓	×
Metal between pipes row 45 (4 down)	✓	×
Drip line row 45	×	×
Drip line row 34	×	×
Drip peg row 34	×	×
Drip peg row 39	×	×
Stanchion row 10, between metal plate and post	✓	×
Large pipes on back wall by door	×	✓
Grey irrigation pipes/rig row 39	✓	×
Stanchion row 39/41, pathway	✓	×
Purple picking crate	✓	×
Purple picking crate	✓	×
Under heating pipe row 42	✓	×
White in crop pipe row 37, inside end	✓	×
White in crop pipe row 38, inside end	✓	×
Metal support for white pipe	✓	✓
Under heating pipe row 47	✓	×
Pipes on glass at end of row 47	✓	×
Centre of concrete path row 43	✓	×
*Taps on water cooler / grower phone	✓	×
*Plant support / plastic jug to catch drips	✓	×
Spray trolley inc. fabric straps	✓	×
Floor of trolley 362	✓	✓
Outside of electrical box row 41	✓	×
Metal plate to hold wires behind stanchion row 41, with holes	✓	×
Waste bin	✓	×
Rubber irrigation pipe	✓	×
Glass joining concrete, end row 41	×	×
Wheels of trolley 362	✓	✓
Small trolley carrying electricals	✓	✓
Glass end of row 28	×	×
Glass in door	✓	×

Outside door handle	✓	✓
Grey pipe floor row 39	✓	×
Wooden pallet	✓	×
Picking crate handle	✓	×
Stanchion row 40, middle or row	✓	×
First aid box	✓	✓
Hand sanitiser by door	×	×

*not available at second visit; / swabbed instead

Site 2

	Result of LAMP assay	
Location swabbed	Pre-clean	Post-clean
Concrete pathway by row 106	✓	✓
Concrete pathway by row 173	✓	✓
Glass at end of 106	✓	×
Glass at end of 105	×	×
Aluminium post at 101	✓	✓
Drip line 173	×	×
Drip line 172	×	×
Drip peg 173	✓	×
Drip peg 172	✓	×
Inside door handle	✓	×
Alcohol gel dispenser	✓	×
Leafing cage 34	✓	✓
Ladder on crop trolley 37	✓	✓
Heating pipe 177 (bottom)	×	✓
Heating pipe 177 (top)	✓	×
Black picking crate	✓	×
Green picking crate	✓	✓
Outside door handle	✓	×
Small electrical panel 161	✓	✓
Electrical panel	✓	✓
Mypex row 173	×	✓
Mypex row 176	×	×
Water cooler	✓	×
Pipes on back wall	✓	×
Between/below slabs 177	✓	×
Spray trolley 2	✓	×
Spray trolley 4	✓	×
Pen from site	✓	×
Aluminium post 1st on right	✓	×
Black de-leafing bin	✓	✓
Glass at end of row 173	✓	×
Metal on floor 173	✓	✓
Hook at end of 173	✓	×

Wheels of leafing cage 17	✓	✓
Trolley 33	✓	✓
Leafing cage 17	✓	×
Plugs/switch at 169	✓	✓
Below gutter 173	✓	×
Fruit trolley (no number)	✓	✓
Fruit trolley (no number)	✓	✓
Concrete pathway	✓	✓
Control box	×	✓
Leafing cage (no number)	✓	×
Plant support bracket	✓	✓
Spray boom	✓	✓
Inside door handle	✓	×
Heating pipe surface	✓	✓
Black picking crate	✓	×
Outside door handle	✓	NT*
Concrete pathway by door	✓	×

*Not Tested, swab missing

Site 3

	Result of LAMP assay	
Location swabbed	Pre-clean	Post-clean
138 Concrete	✓	×
136 Contrete	✓	×
Glass at end of 137	✓	×
Glass at end of 139E	✓	×
Heating pipes Row 135	✓	×
Heating pipes Row 139	✓	✓
Metal floor plate 135	✓	✓
Metal floor plate 139	✓	✓
Grow pipes 141	✓	×
Grow Pipes 142	✓	✓
Green packing crate	✓	✓
Big Black packing crate	✓	×
Blue packing crate	✓	✓
Drip peg half row 130	✓	✓
Drip peg half row 130	✓	✓
Drip line half row 130	✓	✓
Drip line half row 130E	✓	✓
Water cooler small tap	×	×
Water cooler big tap	✓	×
Green waste bin - inside bottom	✓	✓
Moth light, row 131	✓	✓
Picking trolley	✓	×

Crop work trolley floor	✓	✓
Crop work trolley rails	✓	✓
Gutter support 137	✓	✓
Gutter 134	✓	×
Gutter 135	✓	×
Mypex 131	✓	✓
Door handle inside	✓	×
Door handle outside	✓	✓
Ceiling chain 139 2 in	✓	✓
Stanchion 140 end row	✓	✓
Stanchion row 135 6 in	✓	✓
Stanchion row 135 half way	✓	×
Irrigation pipe under gutter row 152	✓	✓
Irrigation pipe under gutter row 129	✓	✓
Electrical trolley charge point 125	✓	✓
Electrical trolley charge point 121	✓	✓
Tag row 138	✓	×
Tag row 142	✓	✓
Hand sanitiser	✓	✓
Whiteboard for jobs	✓	✓
Green button	✓	✓
Green button	✓	✓
Electricals outside	✓	✓
Electric pallet truck folks	✓	✓
Electric pallet truck handles	✓	✓
Electrical switch nearest door	✓	✓
Soap dispenser in wash area - middle	✓	✓
Door and handle and key pad to office	✓	×

