Grower Summary

PE 025

Development and deployment of genotype-specific LAMP assays for monitoring Pepino mosaic virus (PepMV) in tomato

Final *2016*

Disclaimer

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

The results and conclusions in this report may be based on an investigation conducted over one year. Therefore, care must be taken with the interpretation of the results.

Use of pesticides

Only officially approved pesticides may be used in the UK. Approvals are normally granted only in relation to individual products and for specified uses. It is an offence to use non-approved products or to use approved products in a manner that does not comply with the statutory conditions of use, except where the crop or situation is the subject of an off-label extension of use.
Before using all pesticides check the approval status and conditions of use.
Read the label before use: use pesticides safely.

Further information

If you would like a copy of the full report, please email the AHDB Horticulture office (hort.info.@ahdb.org.uk), quoting your AHDB Horticulture number, alternatively contact AHDB Horticulture at the address below.

AHDB Horticulture,
AHDB
Stoneleigh Park
Kenilworth
Warwickshire
CV8 2TL

Tel – 0247 669 2051

AHDB Horticulture is a Division of the Agriculture and Horticulture Development Board.

Project title:	Development and deployment of genotype-specific LAMP assays for monitoring *Pepino mosaic virus* (PepMV) in tomato
Project number:	PE 025
Project leader:	Sarah Mayne, ADAS UK Ltd
Report:	Final Report, September 2016
Previous report:	None
Key staff:	Tim O'Neill, ADAS UK Ltd Matt Dickinson, University of Nottingham; Bethan Warman, University of Nottingham Anna Skelton, Fera Science Ltd
Location of project:	ADAS Boxworth, Cambridgeshire University of Nottingham, Sutton Bonington Fera Science Ltd, Sand Hutton, York Commercial sites
Industry Representative:	Dr Phil Morley, APS Salads/TGA Brian Moralee, Wight Salads, Main Road, Arreton, Isle of Wight
Date project commenced:	1 July 2015
Date project completed:	30 September 2016

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.

.

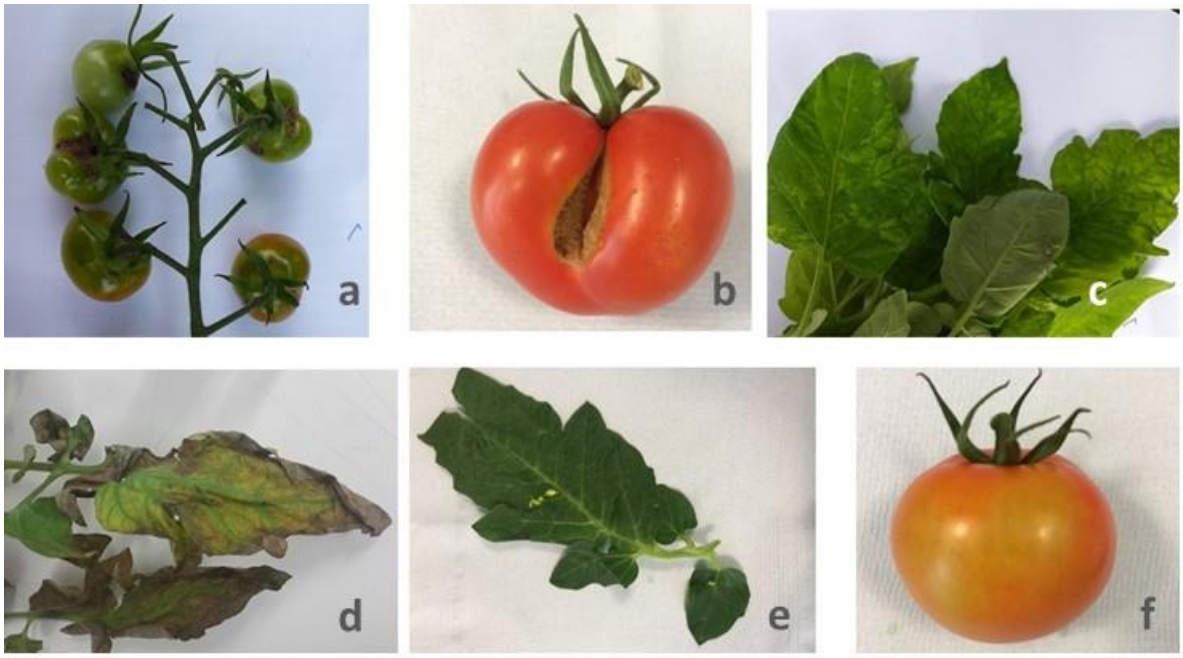

Figure 1. Examples of symptoms observed associated with PepMV infection including splitting of fruit (a, b), chlorotic heads (c), leaf necrosis (d), chlorotic leaf spotting (e), and fruit marbling (f) – 2015 & 2016.

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	Piccolo / Maxifort	Organic	✓	-	-
	07/10/2015			✓	-	-
2	18/09/2015	Roterno / Maxifort	Rockwool	✓	-	✓
	12/10/2015			✓	-	✓
3	15/10/2015	Piccolo / Maxifort	Rockwool	✓	✓	✓
4	08/03/2016	Piccolo / Maxifort	Organic	✓	-	-
	12/04/2016			✓	-	-
5	15/03/2016	Sunstream / Maxifort	Rockwool	✓	-	-
	20/04/2016			✓	-	-
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.

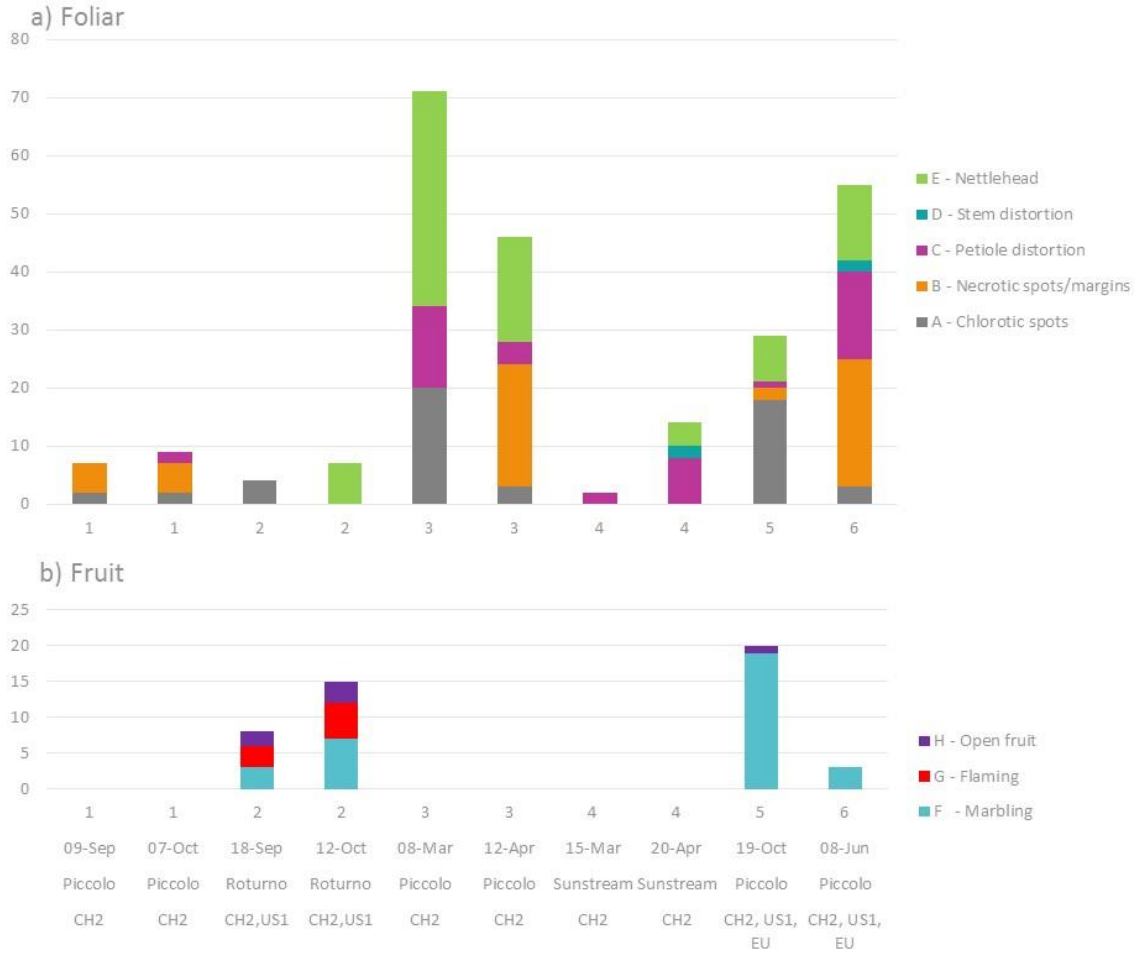

Figure 2. Occurrence (% plants) and type of severe PepMV symptoms (index >2) in tomato crops examined at six UK sites in 2015-2016, a) foliar, b) fruit. The crops at sites 1-4 were each assessed twice, approximately 1 month apart. 100 plants assessed for symptoms and 30 plants tested for PepMV at each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