FACTSHEET 25/16
Protected Edibles

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Project No. PE 025

Pepino mosaic virus of tomato – new results on strains, symptoms and persistence

Pepino mosaic virus (PepMV) is one of the most economically important tomato diseases in the UK. AHDB-funded project PE 025 utilised new molecular assays that can discriminate three PepMV strains in order to increase understanding of symptom severity and further examine disease persistence. This factsheet provides new results on strains present in UK crops, PepMV symptoms, survival of the virus in roots and composted tomato waste, potential locations of contamination after an outbreak and occurrence of the virus in water.

Figure 1. Fruit marbling symptoms due to presence of PepMV

Action points

- PepMV is still notifiable. A suspected outbreak should be reported to the Animal and Plant Health Agency (APHA).
- The LAMP assay indicated the presence of three PepMV strains (CH2, EU and US1) in UK tomato crops in 2015 and again in 2016. Sample testing for PepMV strain (CH2, EU, US1) is available, as a chargeable service, at Fera Science Ltd, Sand Hutton, York YO41 1LZ.
- Biosecurity efforts should be maintained even when PepMV is already present, as introduction of additional strains appears linked to more severe fruit symptom expression.
- Glasshouse end-of-year clean-up and disinfection procedures appear to be successful but the virus may survive in difficult to treat areas, especially electrical equipment, heating pipe supports and trolley wheels (see Table 3).
- The risk of carry-over in roots and soil from one crop to the next appears to be very small where there is at least a six-week gap between successive plantings in soil.
- Aerobic composting to a high temperature is an effective way to eliminate PepMV from crop waste, provided sufficiently high temperatures are reached for a long enough period of time.
- PepMV was detected in reservoir water on one site. Previous work has shown that PepMV can spread between plants in contaminated water and cause disease via root infection. Infested irrigation water could potentially result in widespread infection on a site; it could possibly reintroduce the virus after clean-up. Assess the risk of crop debris, glasshouse condensation water or other potentially infested material contaminating water sources and, where possible, take measures to reduce the risk. Check that water disinfection treatments are operating effectively.

Background

PepMV was first recorded in the UK in 1999, causing a variety of symptoms ranging from slight impacts on growth to severe necrosis, with some cultivars more affected than others. The disease remains one of the most economically important problems affecting tomato production in the UK. Control options are limited, with growers altering growing practices to minimise effect on yield. Thorough hygiene and disinfection between crops has eliminated PepMV on some nurseries, yet on others the virus continues to cause problems each year. In late 2013, a small number of crops infected by PepMV were tested to determine strain. While the majority of samples were positive for the CH2 strain only, currently the predominant strain in Europe, surprisingly two samples tested positive for the US1 strain. The occurrence of mixed strain infections can result in severe symptoms. Several other factors, including variety and stage of crop growth, can affect symptom severity. A new molecular test method (LAMP assay) that can be used to rapidly discriminate between CH2, EU and US1 strains of PepMV is now available. The aim of project PE 025 was to validate the new LAMP assay and then conduct tests to increase understanding of PepMV symptom severity and persistence on nurseries.

LAMP assays for PepMV strain

Primers that had been designed and validated in previous studies overseas were purchased and used in 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, allowing for the identification of the specific strain of PepMV. It was confirmed that each primer was successful in detecting its respective PepMV strain, and that each assay had a different annealing temperature; the latter can be used to check the validity of a test result. The primers were used in 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. 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 on sources of PepMV inoculum within the glasshouse.

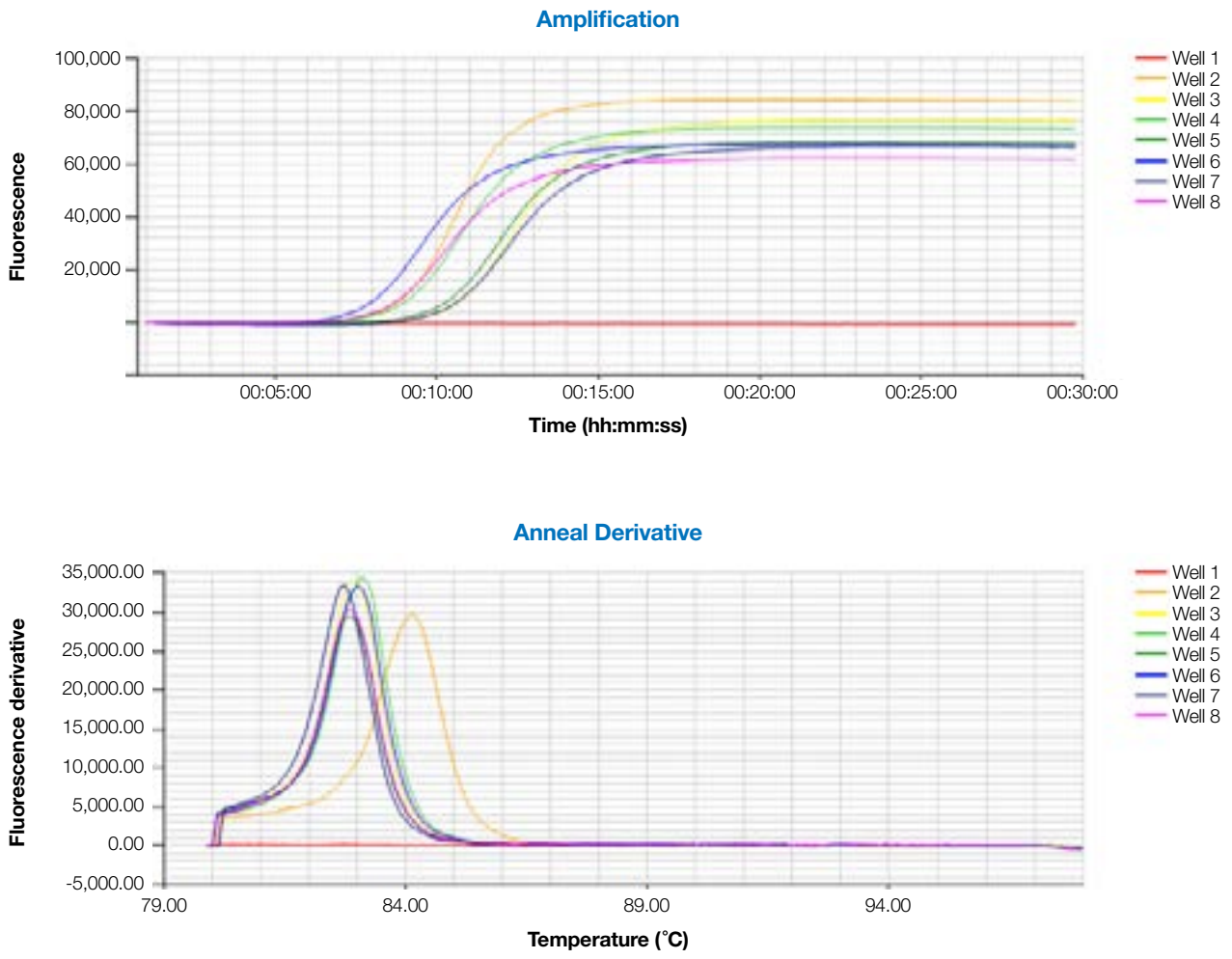

Figure 2. An example output of the LAMP assay when testing for CH2 strain, here showing amplification at around 10 minutes (top) and annealing temperature of around 83°C (bottom). Well 1– negative control; Well 2 – positive control; Wells 3–8 – samples

Table 1. Strains detected in eight UK tomato crops; 30 plants sampled per crop and tested by LAMP assay

Site	Crop	Variety	Date sampled	% samples positive for		
				CH2 strain	EU strain	US1 strain
1	1	Piccolo	9 September 2015	100	0	0
1	1	Piccolo	7 October 2015	100	0	0
2	2	Roterno	18 September 2015	100	0	3.3
2	2	Roterno	12 October 2015	100	0	3
1	3	Piccolo	8 March 2016	100	0	0
1	3	Piccolo	12 April 2016	100	0	0
2	4	Sunstream	15 March 2016	55	0	0
2	4	Sunstream	20 April 2016	10	0	0
3	5	Piccolo	19 October 2015	93	100	100
4	6	Piccolo	8 June 2016	67	100	3
1	7	Lyterno	4 April 2016	100	0	0
1	8	Brioso	31 May 2016	100	0	0

PepMV strains and symptom severity

All three strains were detected. CH2 was most common, both in number of crops affected and proportion of samples testing positive. This strain was frequently detected in asymptomatic plant samples taken from infected crops. The EU and US1 strains were detected in two and three crops, respectively, in mixed infections (Table 1).

Incidence of PepMV symptoms was assessed in six crops. A crop of Roterno infected by both CH2 and US1 strains showed the highest level with 42% of the fruit trusses close to harvest showing symptoms; 10% had severe symptoms. The next two highest levels (39.3% and 11.7%) were on crops of Piccolo with mixed infections of CH2, EU and US1 strains (Figure 3). These results suggest that mixed strain infections can result in more severe fruit symptoms. It is recommended that biosecurity efforts are maintained, even when PepMV is known to be present in a crop. Prevention of additional strains from entering a crop will reduce the risk of new strains being generated through recombination, and appears to reduce the likelihood of severe fruit symptoms. The other factors known to influence symptom severity are discussed in AHDB Technical Review TR-PE 001.

Unlike fruit symptoms there was no correspondence of the number of strains present in a crop with the incidence or severity of foliar symptoms. The highest incidence of foliar symptoms (70% of plants) occurred in a crop of Piccolo in which only CH2 was detected. The main foliar symptoms were chlorotic spots, necrotic leaf margins and nettlehead.

Many of the wide range of symptoms now known to be associated with PepMV infection are shown in Figure 4.

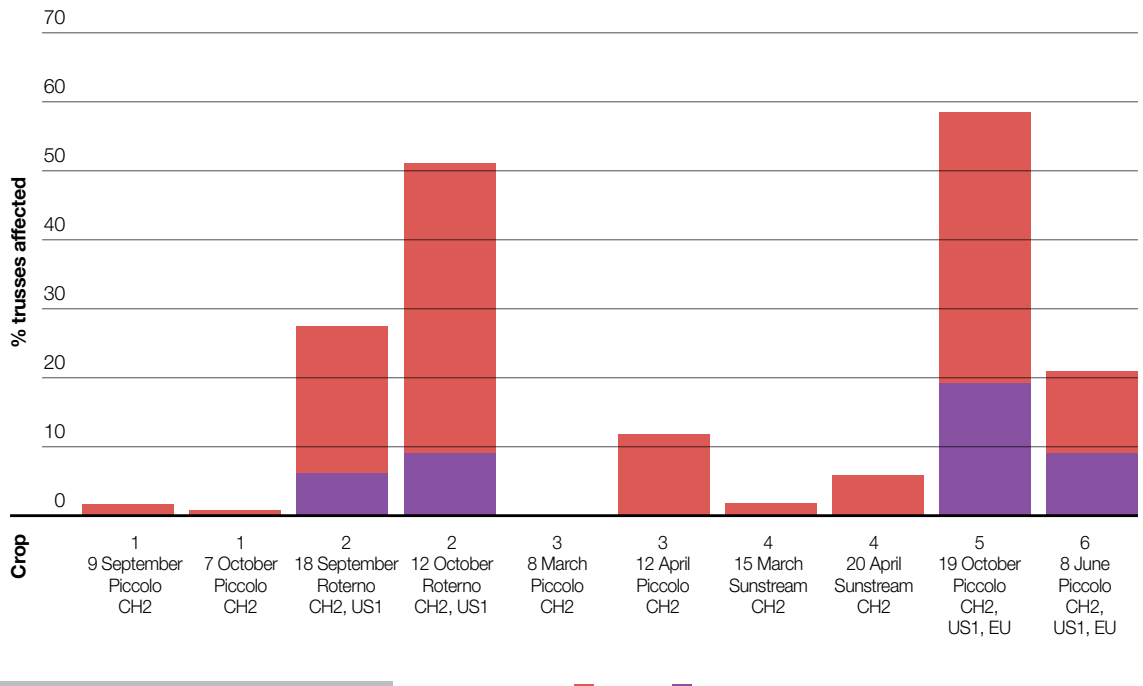

Figure 3. Incidence of PepMV fruit symptoms and strain(s) detected in six UK tomato crops, 2015–2016

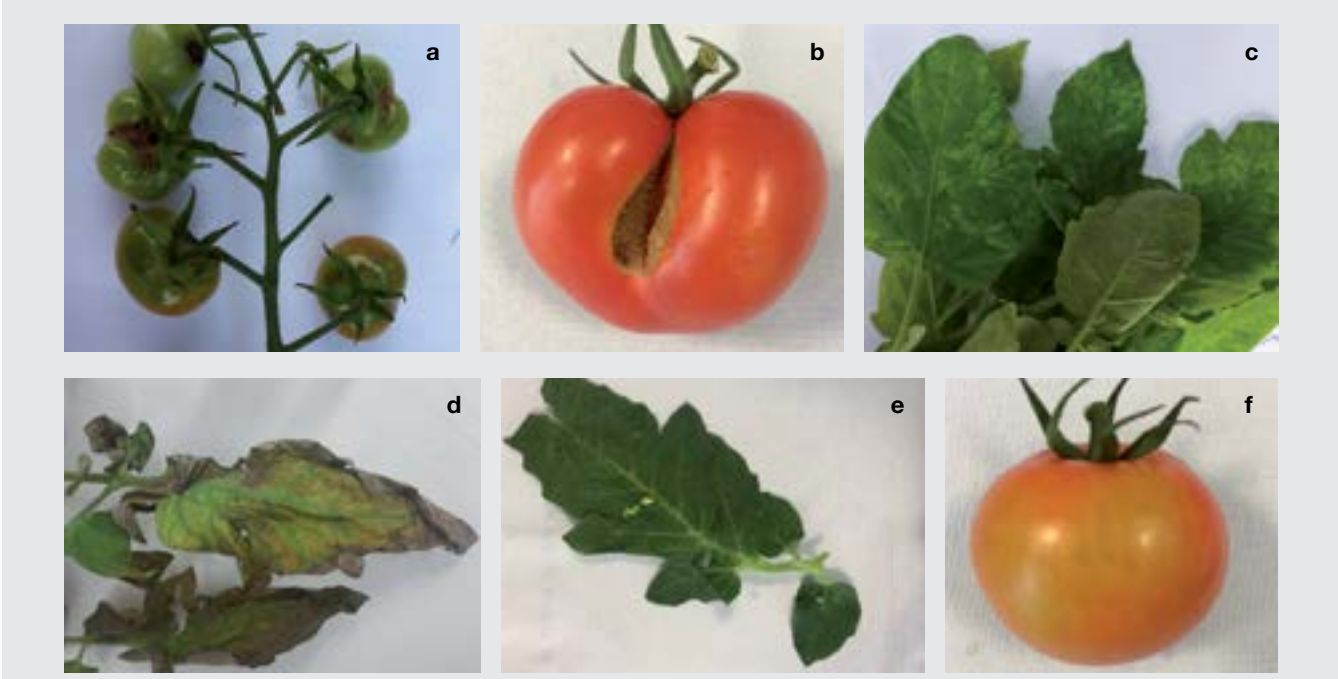

Figure 4. Examples of symptoms observed associated with PepMV infection, including splitting of fruit (a,b), chlorotic heads (c), leaf necrosis (d), chlorotic leaf spotting (e), and fruit marbling (f) – 2015 and 2016

Survival in roots and composted waste

Survival of PepMV in roots and soil was investigated by pulling out known infected tomato plants from an organic crop and testing the small roots that remained in the soil at two-week intervals for six weeks. All large and medium diameter roots were removed with the plant. The smaller root samples remaining in the soil were recovered by soil sieving and then tested by ELISA, and by a sap transmission test onto tomato seedlings to check for virus viability.

PepMV was detected by ELISA in most (28/30) fine root pieces recovered from soil, immediately after plant removal. Sap transmission confirmed viable PepMV in a sample of these root pieces. In subsequent tests, there was a trend for a reduction in detectable PepMV, although the virus was still detected by ELISA at six weeks. However, no viable virus was detected in any of the 13 sap transmission tests on roots recovered at two, four or six weeks after plant removal. Tomato seedling growing-on tests using soil collected at plant removal and six weeks later both proved negative. These results indicate the risk of PepMV remaining in fine roots or soil after crop removal at levels sufficient to result in PepMV infection is low to negligible. Previous work (see PC 181) found that PepMV could survive in roots at transmissible levels for 31 days after plant removal but not eight weeks. As a precaution, it is recommended that large roots should be removed, with the old crop, and the soil cultivated to aid breakdown of fine roots.

Survival of PepMV in tomato waste was examined on a commercial nursery with its own aerobic composting unit. A crop of cv. Piccolo confirmed infected with PepMV (CH2 strain) was pulled out and chipped on 7 November 2015. The chipped crop was stacked in a composting shed, over winter; it was mixed with chipped wood and fresh green leaf from the new crop on 16 March 2016 (Figure 5 and 6), when the composting process began. Windrows had air blown through them and were covered with a breathable Gore-Tex membrane to encourage composting. Samples were taken at intervals and tested by LAMP assay, and sap transmission tests.

At the point of crop removal, all samples of chipped waste examined were found to contain viable PepMV. At the start of composting (16 March), although PepMV was detected by LAMP assay in all samples, no viable virus was detected by sap transmission tests. The proportion of samples testing positive by the LAMP assay declined during March and April (Table 2). None of the samples tested after 7 November were found to contain viable PepMV. The stack of chipped waste awaiting admixture with other materials heated up to 64.3–68.0°C during December–February; the compost windrows achieved temperatures of 51.0–83.6°C during March and April. These results indicate that composting to a high temperature is an effective method for eradication of PepMV from chipped tomato stem waste and deleafing/sideshooting waste.

Virus persistence between crops

Consistent with earlier work (PC 181), on nurseries where PepMV had been established for several weeks, the virus was detected almost everywhere. This included concrete pathways, glass, support wires and aluminium stanchions; irrigation pipes, dripper lines and irrigation pegs; heating pipes and their supports; picking crates and waste bins; trolleys and forklift trucks; doors, water dispensers and alcohol gel dispensers; electrical panels and switches. Levels of detectable PepMV were greatly reduced on nurseries by end-of-crop clean-up and disinfection programmes and no viable PepMV was detected after disinfection. Areas that appeared more difficult to disinfect were trolleys and electrical panels (Table 3). Previous work has shown that PepMV in sap expressed from leaves can survive only a relatively short time, for example, up to 14 days at 15°C. Survival was less (2–4 days) at high temperatures (20–25°C). The greater risk of carry-over between crops is likely to arise from any fruit or leaf fragments that are missed during clean-up, or from volunteer tomato seedlings that germinate from fallen fruit.

Figure 5. Three rows encompassing the composting of tomato waste at a commercial site. Compost is mixed and put in rows (left), the row is then turned (middle), before finally being left out to dry (right)

A method developed at the National Institute of Biology (NIB) Slovenia for filtering water to enable virus testing was used on three nurseries. PepMV was detected in reservoir water in April 2016 on one nursery. This could represent a pathway for rapid spread of PepMV through crops, and could possibly reintroduce the virus to a new crop after clean-up. As a precaution, seek to minimise the risk of crop debris, glasshouse condensation water or other potentially infested material entering reservoirs or other stored water sources. Survival of PepMV in water has not been thoroughly examined; it is reported to survive up to three weeks in nutrient solution. Pasteurisation (at least 30 seconds at 95°C) was shown to successfully eliminate PepMV on one nursery where drainage water was infested.

Figure 6. Compost taken from chipped tomato waste after four months (left) in comparison to the control treatment, where freshly chipped tomato waste was held at a steady 24°C for four months (right)

Table 2. Detection of PepMV in chipped tomato waste and compost produced from this waste

Date tested	PepMV detection method (% positive)					
	LAMP assay		Sap Inoculation		Post-sap inoculation ELISA	
	Compost	Control	Compost	Control	Compost	Control
7 November 2015	NT	NT	100	100	100	100
16 March 2016	100	NT	0	0	0	0
23 March 2016	80	100	0	0	0	0
30 March 2016	30	90	0	0	0	0
5 April 2016	70	100	0	0	0	0
12 April 2016	70	100	0	0	0	0
26 April 2016	10	NT	NT	NT	NT	NT

NT – not tested

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. Note that, although PepMV was detected at some locations after clean-up, none of the samples tested proved viable

Area swabbed	Proportion of samples positive for PepMV before (pre) and after (post) clean-up and 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

Further information

Useful AHDB project reports

Factsheet 11/01: 'New results on pepino mosaic of tomato'.

Factsheet 20/03: '*Pepino mosaic virus* of tomato – new results on virus persistence and disinfection'.

Technical Review PE 001 (2014) *Pepino mosaic virus*: strains, symptoms and cross-protection.

PE 025 Development and deployment of strain-specific LAMP assays for monitoring *Pepino mosaic virus* (PepMV) in tomato.

Acknowledgements

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