

**Title** Protected tomato: sources, survival and disinfection of Pepino mosaic virus (PepMV)

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The results and conclusions in this report are based on a series of experiments 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.

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## **Grower Summary**

### **Headline**

Pepino mosaic virus (PepMV) can be eliminated from solid surfaces by chemical disinfection, high-pressure hot water washing and by natural decay with time.

### **Background and expected deliverables**

Pepino mosaic virus (PepMV) was first reported in the UK in a tomato crop in January 1999 and has been confirmed in further crops each subsequent season. It is a mechanically transmitted virus in Potex (Potato virus X (PVX)) group and is extremely contagious. Hands, clothing and tools are believed to be the primary means of spread. Nursery experience indicates there is a significant risk of carryover once a nursery is affected.

Infection results in a range of symptoms that commonly include leaf mosaic and bubbling, a pale green spiky head to the plant, angular yellow spots on leaves, plant stunting and marbling. Visibly affected fruit are unmarketable and yield of Class I fruit may be reduced by around 10%.

The expected deliverables from this project are:

- Knowledge of where the virus may occur on a nursery after an outbreak.
- Information on survival of the virus on solid surfaces under different environmental conditions.
- Information on survival of the virus in roots in soil
- Identification of chemical disinfectants fully effective against the virus
- Efficacy of high pressure, hot water washing as a method of disinfecting the glasshouse structure and plastic trays.

## **Summary of the project and main conclusions**

### **Persistence on a nursery**

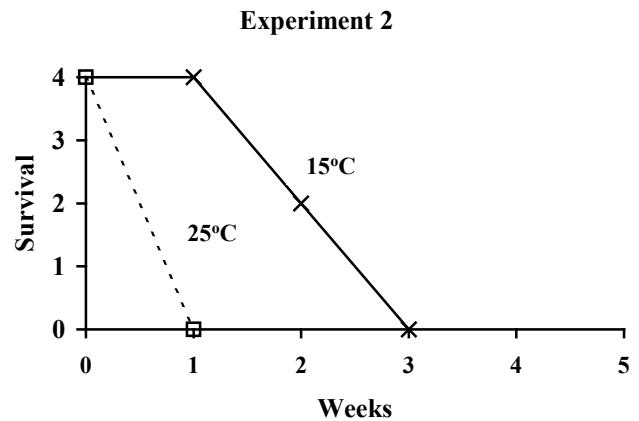
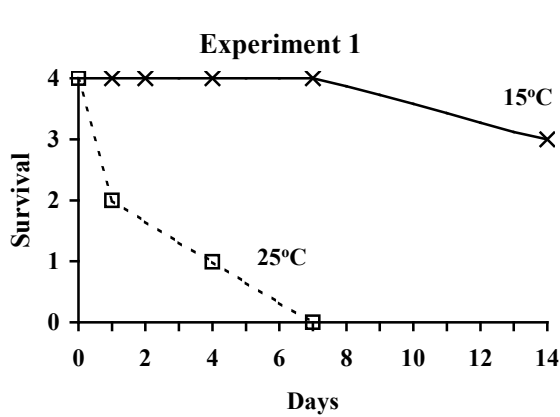
Monitoring on two affected nurseries in August 2000 revealed PepMV at transmissible levels on various surfaces and equipment. Contaminated surfaces included concrete pathways, polythene floor covering, picking trolleys, waste containers, irrigation lines, drip pegs, aluminium stanchions, wooden stakes at ends of rows and run-off solution. Detection of the virus was more frequent in a house where the disease had been present for several months than in a house only affected for a few weeks. Volunteer tomato seedlings collected from within houses at this time also tested positive. At one of the nurseries, following an end-of-season clean-up and disinfection with trisodium orthophosphate (TSOP), the virus was not detected at transmissible levels in November 2000. However, ELISA tests indicated the occurrence of virus, or virus remnants, on some surfaces including concrete pathways, new polythene floor covering, heating pipe stands, within drip nozzles, concrete

stanchion bases and on uncleaned picking crates and containers. More significantly, PepMV was detected in fruit and stem debris found within one 'clean' house.

### Survival on hard surfaces

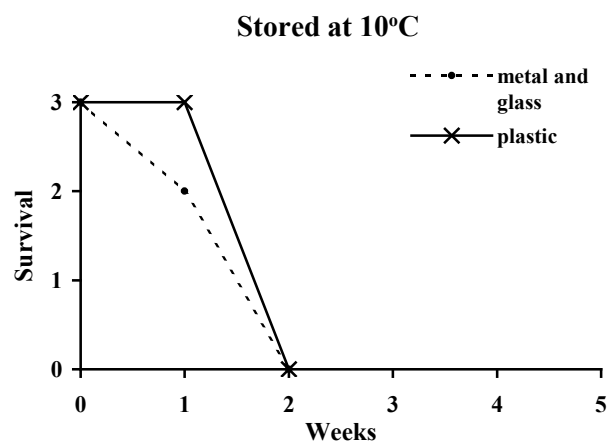
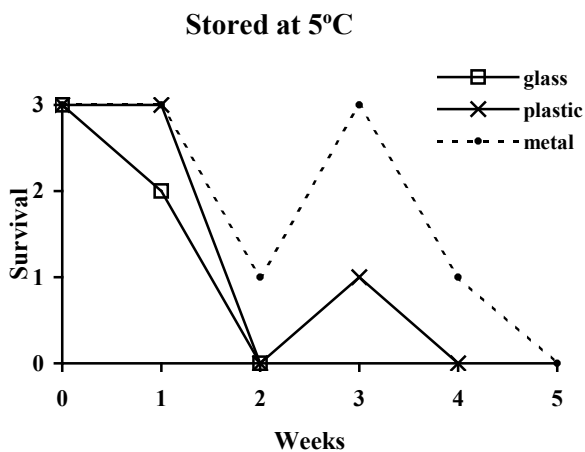
#### On glass at warm temperatures (leaf sap)

- Survival was greater at 15°C than 25°C.
- No transmissible PepMV was detected after 3 weeks at 15°C.



#### On plastic, metal and glass at cool temperatures (leaf and fruit sap)

- Survival was greater at 5°C than 10°C
- At 5°C, transmissible PepMV was detected after 4 weeks but not after 5 weeks.



## Survival in roots

PepMV was confirmed in tomato roots to at least 30-cm depth. Virus at transmissible levels was detected in roots 31 days after plants were cut-off at soil level, but not after 57 days. Work outside this project indicates the risk of transmission from infected root pieces in the soil to tomato plants is low.

## Chemical disinfectants

### Long exposure time (1 hour)

Nine chemical disinfectants tested at their recommended rates (Table 1) were effective in disinfecting five surfaces (aluminium, concrete, glass, plastic and polythene) deliberately contaminated with PepMV in tomato leaf sap. Disinfection was successful after 1 hour. Effective disinfection frequently took longer, up to 24 hours, or was not fully effective, when products were tested at reduced rates. The disinfectant which performed best at all dilutions (Horticide) was tested again for disinfection of surfaces deliberately contaminated with PepMV in juice from infected tomato fruit. Results showed that it performed less well at disinfecting PepMV in tomato juice.

**Table 1: Summary of disinfectants tested and found effective against PepMV (1 hour contact time) on various surfaces**

Disinfectant	Rate used <sup>a</sup>	Surface				
		Aluminium	Concrete	Glass	Polythene	Plastic
Ben Glucid	2%	✓	✓	✓	✓	✓
	0.5%	X	X	✓	✓	✓
Glucid	2%	✓	✓	✓	✓	✓
	0.5%	✓	✓	✓	✓	✓
Horticide	1:25	✓	✓	✓	✓	✓
	1:100	✓	✓	✓	✓	✓
Jet 5	1:125	✓	✓	✓	✓	✓
	1:400	X	✓	✓	✓	✓
MennoFlorades	4%	✓	✓	✓	✓	✓
	1%	X	X	X	✓	X
Panacide M	0.5%	✓	✓	✓	✓	✓
	0.125%	✓	✓	✓	✓	✓
Sodium hypochlorite	400 ppm	✓	✓	✓	✓	✓
	100 ppm	✓	✓	✓	✓	✓
TSOP	10%	✓	✓	✓	✓	✓
	2.5%	X	✓	✓	✓	✓
Virkon S	1%	✓	✓	✓	✓	✓
	0.25%	✓	X	✓	✓	✓

<sup>a</sup> Maximum label rate and one quarter of that rate (See Annual Report of June 2001 for more detailed results)

✓ = fully effective; x = not fully effective

Spraying surfaces contaminated with PepMV from tomato leaf with water also reduced the level of PepMV, although the virus was still detectable on some surfaces after 24 hours. However, when surfaces were contaminated with PepMV in juice from infected tomato fruit, water spray alone had very little effect in reducing levels of PepMV.

#### Short exposure time (1-30 minutes)

The most effective products that quickly disinfected a surface deliberately contaminated with PepMV were Virkon S and Unifect G (Table 2).

**Table 2: Summary of disinfectants tested and found effective against PepMV with a short contact time (1 to 30 minutes)**

Disinfectant	Rate used	Leaf sap			Fruit juice		
		1	5	30 mins	1	5	30 mins
Jet 5	1:125	(✓)	X	X	X	X	X
Sodium hypochlorite (5% chlorine) <sup>a</sup>	1:10	(✓)	(✓)	(✓)	(✓)	X	X
Panacide M	0.5%	X	X	X	X	X	X
Virkon S	1%	✓	(✓)	✓	✓	✓	✓
Unifect G	1:25	✓	✓	✓	✓	✓	✓

<sup>a</sup>Equates to 5,000 ppm available chlorine

✓ = fully effective, (✓) = partially effective, X = not effective

#### Food grade disinfectants

Disinfectants recommended for use in food areas (e.g. canteens) and on hands differ from those recommended for use in empty glasshouses. Two handwash soaps and a table spray were tested for their efficacy against PepMV. These were 'Sensisept' handwash soap (ai chlorheximide), 'Med' handwash gel (ai alcohol) and 'Delladet' table spray (ai QAC). For contaminated hands, washing with Sensisept soap followed by Med gel was fully effective, and thoroughly washing in water followed by Med gel gave a large reduction. Washing in water alone gave no reduction. For contaminated Formica, Delladet table spray proved ineffective.

#### **High pressure, hot water washing**

The preferred, commercially acceptable method for cleaning plastic trays on tomato nurseries is with water and a detergent, not with chemical disinfectants. Grower experience indicates that a high temperature high-pressure water wash may be effective. Equipment supplied by BritClean (UK) Ltd of Stoke on Trent was tested on rigid plastic deliberately smeared with squashed tomato fruit and PepMV. Sap transmission tests showed that the following treatments were effective: manual washing for 3 mins at 60°C or above; pressure washing at 1300 psi for 3 seconds at 50°C at the nozzle and above.

## Financial benefits

As this disease is new to Europe and to protected tomato crops, there was relatively little knowledge on how best to control it when the project commenced. Best-practice recommendations are currently based on the results of experiments with related viruses (e.g. PVX, ToMV). Results from this work will substantially increase growers' knowledge of:

- 1) potential sources of PepMV in an affected glasshouse.
- 2) the risk of the virus surviving on different surfaces and at different temperatures and in soil between crops.
- 3) the effectiveness of chemical disinfection and washing treatments.

An outbreak of PepMV in a tomato crop can result in substantial financial cost. Control is effected primarily by removal of plants. In the early stages of the disease, the practice is to remove all plants in the affected area, together with a surrounding *cordon-sanitaire*. Statutory conditions are imposed by PHSI at sites where PepMV is confirmed in England. Losses result from:

- cost of removal and disposal of infected plants.
- cost of new plants and rockwool slabs.
- a delay before the replanted crop comes into production.
- cost of staff time and consumables (e.g. disposable overclothes) in efforts to prevent spread to other houses.
- reduction in marketable fruit yield
- potential inability to maintain supply to the customer (supermarket contracts).

It is estimated that losses on three UK nurseries affected in 1999 totaled well in excess of £200,000. There have been several further outbreaks each year since then. If the control measures identified here prevent the recurrence of PepMV on a nursery after an outbreak, there is a potential financial benefit of around £70,000 per nursery, based on the above estimates.



## Action points for growers

### Persistence on a nursery

1. Many surfaces in a glasshouse were found to be contaminated following an outbreak of PepMV. *Adopt a strict hygiene protocol to minimise the risk of rapidly spreading the disease (see article in Grower, 7 December 2000, pages 20-22, for details).*
2. While PepMV is relatively short-lived, it can persist in dried sap from a few days to a few weeks depending on the temperature in a glasshouse. *Movement of staff and equipment between houses risks spreading PepMV. Change to new coveralls, gloves and overshoes when moving between an infected and healthy crop; keep separate equipment (e.g. trolleys, boxes) for each house. If practical, avoid entering more than one house on the same day.*
3. Good clean-up and disinfection programmes can eradicate the disease. *Rigorous attention to removal of fallen fruit and all other crop debris is essential at crop turn-around.*

### Survival on surfaces and in soil

4. PepMV survives longest in cool conditions – for up to 4 weeks at 5°C. At 25°C, survival was for less than 1 week. *After an outbreak of PepMV, it is suggested that an empty glasshouse and equipment be maintained free of contact with tomatoes for an appropriate period to allow natural decline of the virus in dried sap to zero (e.g. at least 10°C for 3 weeks, or 25°C for 1 week) before the new crop is brought into the house. Consider closing the glasshouse house on sunny days at crop turn-around to raise the temperature.*
5. Although PepMV can occur in tomato roots in soil to at least 30 cm depth, the risk of transmission to new plants appears to be low. *Nevertheless, it is recommended that after an outbreak of PepMV in a soil – grown crop, as much root as possible is removed and that the soil is cultivated at least twice before re-planting to encourage root decay.*

### Transmission from seed

6. Work outside this project indicates PepMV can occur on the outside of tomato seed and transfer to the resultant plant if seed cleaning is poor. *The use of acid-extracted seed, and seed disinfection, are reported to be effective ways of eliminating this risk.*

### Disinfection

7. Chemical disinfectants can be harmful to operators. For example, products containing glutaraldehyde (e.g. Ben-Glucid, Glucid, Horticide, Unifect G) may cause burns and sensitisation by skin contact. *Read and carefully follow the directions for use and the safety precautions on the product label.*

8. Chemical disinfectants shown to be effective in preventing transmission of PepMV when used at their recommended rate for a one hour period are: Ben-Glucid, Glucid, Horticide, Jet 5, Menno-Florades, Panacide M, sodium hypochlorite, TSOP and Virkon S. *Choose a disinfectant most appropriate for the particular use and according to the other tomato pathogens which are a target for disinfection on your nursery.*
9. For quick disinfection, Unifect G and Virkon S were found effective against PepMV in both leaf sap and tomato juice after just one-minute contact time when used at their recommended rates. Note that Horticide and Unifect G are listed as having the same chemical composition (i.e. they appear to be identical products under different names).
10. In a test with Horticide at the recommended rate, PepMV was more difficult to decontaminate in fruit sap than in leaf sap. *Pay particular attention to cleaning and disinfection of equipment contaminated with squashed fruit.*

#### Washing

11. Washing hands with Sensisept soap followed by rubbing with Med gel, or thoroughly washing in water followed by Med gel, will reduce the risk of spreading PepMV on hands. Simply washing hands in water, or rubbing dirty hands with Med gel were not effective. *A strict hand-washing protocol needs to be followed if spread of PepMV from contaminated hands is to be prevented.*
12. High pressure, hot water washing (3 sec at 50°C and 1300 psi) was effective for the removal of PepMV from rigid plastic trays contaminated by PepMV in dried sap. Manual washing for 3 minutes at 60°C was also effective. Simply washing with cold water significantly reduced transmission from surfaces contaminated with PepMV in leaf sap but not in fruit sap. *Do not rely on chemical disinfection alone; review your glass and equipment washing procedures.*

#### PepMV in water

13. PepMV was found at transmissible levels in run – off solution. *After an outbreak of PepMV, do not re-circulate run – off solution unless it is effectively disinfected.*

#### Resistant varieties

14. PepMV has been confirmed in a wide range of tomato varieties. *There is no evidence, at present, of varietal resistance.*

**Please refer to HDC factsheets 12/00, 11/01 and 20/03 for further information on pepino mosaic virus disease of tomato.**