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

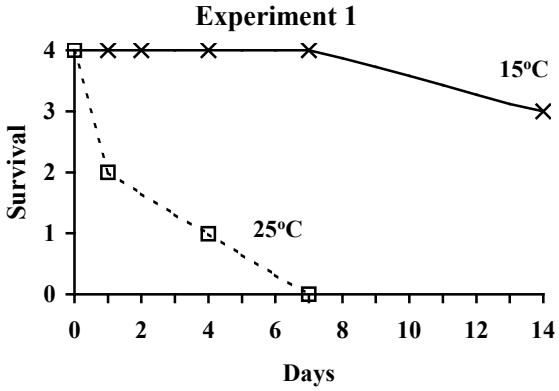
Monitoring on two affected nurseries in August 2000 revealed PepMV at transmissible levels on various surfaces and equipment. Contaminated surfaces included concrete pathways, polythene floor covering, picking trolleys, waste containers, irrigation lines, drip pegs, aluminium stanchions, wooden stakes 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

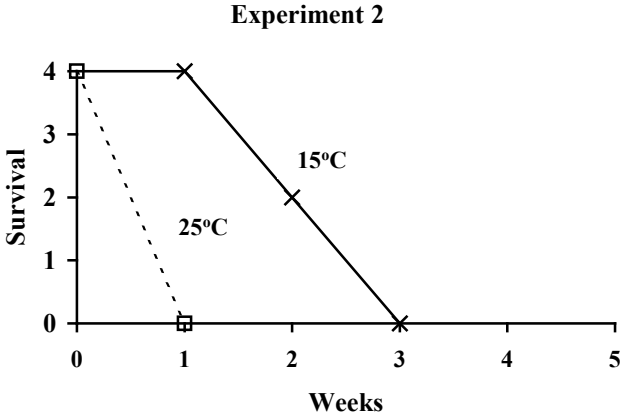
stanchion bases and on uncleaned picking crates and containers. More significantly, PepMV was detected in fruit and stem debris found within one 'clean' house.

Survival on hard surfaces

On glass at warm temperatures (leaf sap)

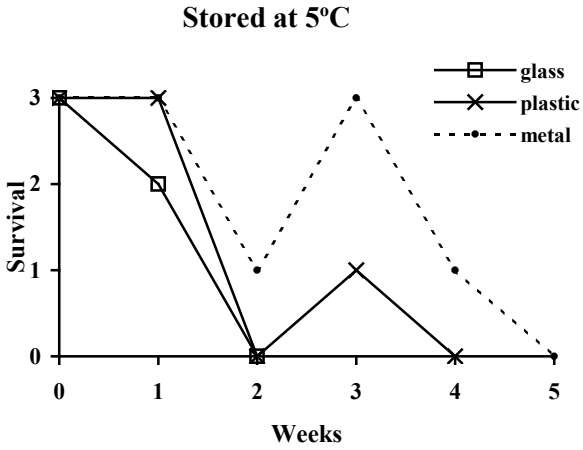
- Survival was greater at 15°C than 25°C.
- No transmissible PepMV was detected after 3 weeks at 15°C.

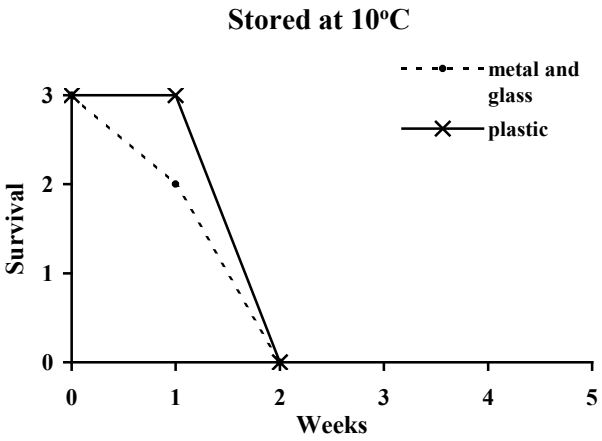




On plastic, metal and glass at cool temperatures (leaf and fruit sap)

- 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

		Surface				
Disinfectant	Rate used [a]	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	✓	✓	✓

[a] 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)[a]	1:10	(✓)	(✓)	(✓)	(✓)	X	X
Panacide M	0.5%	X	X	X	X	X	X
Virkon S	1%	✓	(✓)	✓	✓	✓	✓
Unifect G	1:25	✓	✓	✓	✓	✓	✓

[a]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 bought 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.

Science Section

Introduction

Pepino mosaic virus suddenly and seriously affected protected tomato production in the UK in 1999. The virus is mechanically transmitted and appears to be extremely contagious. Reports from Holland indicate a significant risk of carryover between seasons once a nursery is affected. Accurate information is urgently required to minimise risk of further outbreaks of this disease. In the longer term the most effective method of control is likely to be by the use of resistant varieties, as has been achieved with the $Tm2^2$ gene for control of tomato mosaic virus (ToMV). In the short-term however, we need to identify the most effective precautions to limit spread and treatments to eradicate the virus.

The virus was first described in pepino in Peru in 1980 during a survey of weeds to find natural hosts of potato virus disease. Work at the time showed that the virus was transmitted by plant contact and not by aphids. Sap from infected *Nicotiana glutinosa* plants remained infective for at least 3 months at 20ºC and for 6 months in desiccated *N. glutinosa* leaves. The virus was found to have a narrow host range, infecting 30 out of 32 species of Solanaceae tested, all systemically and *Cucumis sativus* (cucumber) and *Tetragona expansa* in inoculated leaves only. It failed to infect 13 species in 6 other families.

In Holland, a working group on PepMV was established and initial tests with tomato indicated:

- dried leaves are still infective
- the virus concentration in roots is very high
- the virus can survive in plant sap at 20 ºC under dry conditions for 1 day, not 4 days (survival under humid conditions is not known)
- disinfectants based on hydrogen peroxide do not work with a short contact time

Work funded by HDC in 2000/2001 (Project PC 181)

In the initial phase of this project (July 2000 – June 2001) work was done to:

- identify common sources of the virus on affected nurseries
- investigate survival of the virus under different environmental conditions (temperature, humidity, light)
- evaluate selected chemical disinfectants against PepMV
- summarise new UK and overseas research results on the disease

Results are presented in full in the June 2001 annual report for PC 181 and are summarised in the grower summary of this report.

New HDC funded work 2002/2003 (Project PC 181)

In the extension of this project (October 2002 – July 2003), work was undertaken to:

- Evaluate short duration chemical disinfection treatments
- Evaluate soaps and sanitisers for use in food areas
- Evaluate the efficacy of washing for decontaminating surfaces
- Investigate survival of PepMV at cool temperatures (5-10°C)

1. Evaluation of short duration disinfection treatments

Introduction

In year 1 of the project (July 2000-June 2001), a wide range of disinfectants were shown to have good activity against PepMV when contaminated surfaces were exposed to disinfectant for 1 hour or more. Contact times of less than 1 hour were not tested. Information on the relative efficacy of disinfectants after a short period is required in order to determine if spray treatments which dry quickly are effective (disinfectants are generally considered ineffective once they have dried).

Methods

PepMV-infected tomato leaf was collected two-weeks after inoculation and confirmed positive by ELISA. The infected leaves were ground up in phosphate buffer (1:5 dilution) and inoculated onto plastic trays and allowed to dry.

Five disinfectants were used (Virkon S, Unifect G, Panacide M, Jet 5 and sodium hypochlorite) and water as a control. Each disinfectant was sprayed onto the PepMV infected trays and swabs were taken with cotton buds dampened with phosphate buffer after 1 minute, 5 minutes and 30 minutes. The cotton buds were immediately rubbed gently onto the leaves of *Nicotiana benthamiana* plants (wrapped individually to avoid cross contamination) to test the viability of the virus.

This work was repeated using trays inoculated with juice from PepMV-infected tomato fruit.

After 1 week the indicator plants were tested individually by ELISA for PepMV.

Results and discussion

The results achieved for leaf and fruit sap are given in Tables 1.1 & 1.2 respectively. Virkon S and Unifect G were effective after just one minute contact time on both leaf sap and fruit juice. The other disinfectants were not effective even after 30 minutes contact time.

Table 1.1 Survival of PepMV in tomato leaf sap on plastic trays after treatment with the different disinfectants.

Treatment	Rate	No. of indicator plants (out of 5) positive for PepMV		
		1 minute	5 minutes	30 minutes
Virkon S	1%	0	1	0
Unifect G	1:25	0	0	0
Panacide M	0.5%	3	5	4
Jet 5	1:125	1	3	3
Sodium hypochlorite (5% available chlorine)	1:10	1	2	2
Water	-	5	5	5

Table 1.2 Survival of PepMV in tomato fruit sap on plastic trays after treatment with the different disinfectants

Treatment	Rate	No. of indicator plants (out of 5) positive for PepMV		
		1 minute	5 minutes	30 minutes
Virkon S	1%	0	0	0
Unifect G	1:25	0	0	0
Panacide M	0.5%	3	5	4
Jet 5	1:125	5	5	5
Sodium hypochlorite (5% available chlorine)	1:10	1	4	3
Water	-	5	5	5

2. Disinfectant soaps and sanitisers for use in food areas

Introduction

Imported fruit affected by PepMV is now considered to be an important source of infection for UK nurseries. There is a particularly high risk where imported fruit are packed on the same site as a UK production nursery, and staff share a common canteen facility. Disinfectant products 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, all recommended as suitable for the purpose, were tested.

Methods

A mixture of tomato leaves and fruit infected with PepMV was ground up in phosphate buffer (1:5 dilution) and used to inoculate hands and a Formica surface. Three different disinfectants were tested:

'Sensisept' handwash soap (active ingredient chlorheximide)
'Med' handwash gel (active ingredient alcohol)
'Delladet' table spray (active ingredient QAC)

The hands were washed under running water using either 'Sensisept', 'Delladet' (with water) or water alone (as a control). The 'Med' was applied to hands after washing (see table 2.1 for the different treatments). The Formica was sprayed with the different disinfectants and then rubbed several times with a cloth (see Table 2.2). As a control, Virkon S was also tested. After washing, swabs were taken with cotton buds and were used to inoculate *N. benthamiana* indicator plants. The cotton buds were then placed individually into a small amount of phosphate buffer, which was tested for PepMV by ELISA. After 1 week, the indicator plants were also tested by ELISA.

As a control, swabs were taken from unwashed inoculated hands and Formica, to show the viability of the virus.

Results and discussion

The results achieved for the hands and Formica are given in Tables 2.1 and 2.2 respectively.

For contaminated hands, washing with Sensisept soap reduced transmission of PepMV, and when followed by the use of Med handwash gel transmission was reduced to zero. Simply washing in water gave no reduction in transmission of the virus but there was a large reduction when washed hands were subsequently rubbed with Med handwash gel. Rubbing unwashed hands with Med handwash gel was ineffective. Delladet table spray was poor when used on hands.

On a contaminated Formica surface, the Delladet table spray was tested at three rates and all proved ineffective. Washing with water + detergent, or with neat Sensisept, were also ineffective.

Based on these results, washing hands with Sensisept soap followed by Med gel, or thoroughly washing in water followed by Med gel, would appear to be suitable washing treatments to minimise the risk of spreading PepMV on hands.

No effective treatments were found to remove PepMV infected tomato sap from a Formica surface, typical of that found in canteens.

Table 2.1 Survival of PepMV in tomato sap (fruit and leaf) on hands after washing with the different disinfectants

Treatment	No. positive for PepMV by ELISA (out of 3 reps)	
	Swabs	Indicator plants
Untreated, no Med	3	3
Untreated + Med	3	2
Water, no Med	3	3
Water + Med	1	0
Sensisept, no Med	0	1
Sensisept + Med	0	0
Delladet, no Med	0	3
Delladet + Med	1	0

Table 2.2 Survival of PepMV in tomato sap (fruit and leaf) on a Formica surface after washing with different disinfectants.

Treatment	No. positive for PepMV by ELISA (out of 3 reps)	
	Swabs	Indicator plants
Untreated (dry)	3	3
Water + detergent	3	3
Sensisept (neat)	3	2
Delladet 0.5%	3	3
Delladet 2.5%	3	3
Delladet 5.0%	3	3
Virkon S 1% (control)	0	0

3. Efficacy of washing for decontaminating surfaces

Introduction

It is not approved commercial practice to treat the rigid plastic picking trays used on tomato nurseries with disinfectants. The preference is for physical cleaning with water and soap. Grower experience indicates that such treatment may be effective against PepMV but firm evidence is lacking.

Methods

Tomato leaves infected with PepMV and tested positive by ELISA, were ground up in phosphate buffer (1:5 dilution) and used to inoculate areas 10-15 cm in diameter on both the in and out sides of rigid plastic trays. The sap was allowed to dry before the trays were either washed manually or washed using a high pressure washer supplied by Britclean (UK) Ltd, Stoke on Trent. No detergents or disinfectants were used. In total 7, different treatments were used (see tables 3.1 and 3.2). Two trays were used for each treatment, with 2 swabs taken from each tray. Cotton buds dampened in phosphate buffer were used to take swabs from the inoculated areas and to inoculate *N. benthamiana* indicator plants. These were individually wrapped to avoid cross contamination.

As a control, swabs were taken from a tray that had not been washed and were inoculated onto indicator plants, to show the viability of the virus.

The above was repeated with trays contaminated with fruit infected with PepMV.

After 1 week the indicator plants were tested individually by ELISA for PepMV.

Results and discussion

The results achieved for leaf and fruit sap are given in Tables 3.1 and 3.2 respectively.

Manually washing trays, with the aid of a dishwasher, with water at 60°C or above for 3 minutes was fully effective against PepMV in both dried leaf sap and dried fruit juice. Treatment at 50°C was not effective. Using a high pressure hot water washer treatment at 50°C or above, at 1300 psi, was fully effective in just 3 seconds.

Table 3.1 Survival of PepMV in tomato leaf sap on rigid plastic trays after washing manually or using a pressure washer, at different temperatures.

Treatment	No. of indicator plants (out of 4) positive for PepMV.
3 mins at 50ºC, washing manually	1
3 mins at 60ºC, washing manually	0
3 mins at 70ºC, washing manually	0
3 secs at 50ºC & 1300 psi	0
3 secs at 60ºC & 1300 psi	0
3 secs at 70ºC & 1300 psi	0
20 secs at 50ºC & 1300 psi	0
Control	4

Table 3.2 Survival of PepMV in tomato fruit sap on rigid plastic trays after washing manually or using a pressure washer, at different temperatures.

Treatment	No. of indicator plants (out of 4) positive for PepMV.
3 mins at 50ºC, washing manually	2
3 mins at 60ºC, washing manually	0
3 mins at 70ºC, washing manually	0
3 secs at 50ºC & 1300 psi	0
3 secs at 60ºC & 1300 psi	0
3 secs at 70ºC & 1300 psi	0
20 secs at 50ºC & 1300 psi	0
Control	4

4. Survival of PepMV at cool temperatures

Introduction

In year 1 of the project we demonstrated that PepMV dried on a glass surface survives for 2 days but not 7 at 25^0 C and for 14 days but not 21 days at 15^0 C. At crop turn-around in the autumn, glasshouses are usually unheated and trolleys, boxes and other equipment may be stored outside while crop removal and glasshouse cleaning is in progress. It is prohibitively expensive to heat the glasshouse to 25^0 C for, say, 7 days prior to arrival of the new crop to ensure destruction of PepMV. But exposure to a lower temperature for a longer period may be sufficient. Information is required on the survival of PepMV on surfaces at cool temperatures (e.g. 5-10^0 C). This information would also be useful for potential survival in cold stores.

Methods

Tomato plants infected with PepMV were ground up in phosphate buffer (1:5 dilution) and the sap was spread onto plastic trays, glass slides and metal (aluminium) surfaces. These were then placed in 2 unilluminated controlled environment cabinets both kept at 80% relative humidity. One was set at 5ºC and the other at 10ºC. At 2 days, 7 days and then at weekly intervals up to 7 weeks, swabs were taken from the different surfaces, with cotton buds soaked in phosphate buffer and inoculated onto *N. benthamiana* indicator plants. After 7 days the indicator plants were tested by ELISA for PepMV.

Results and discussion

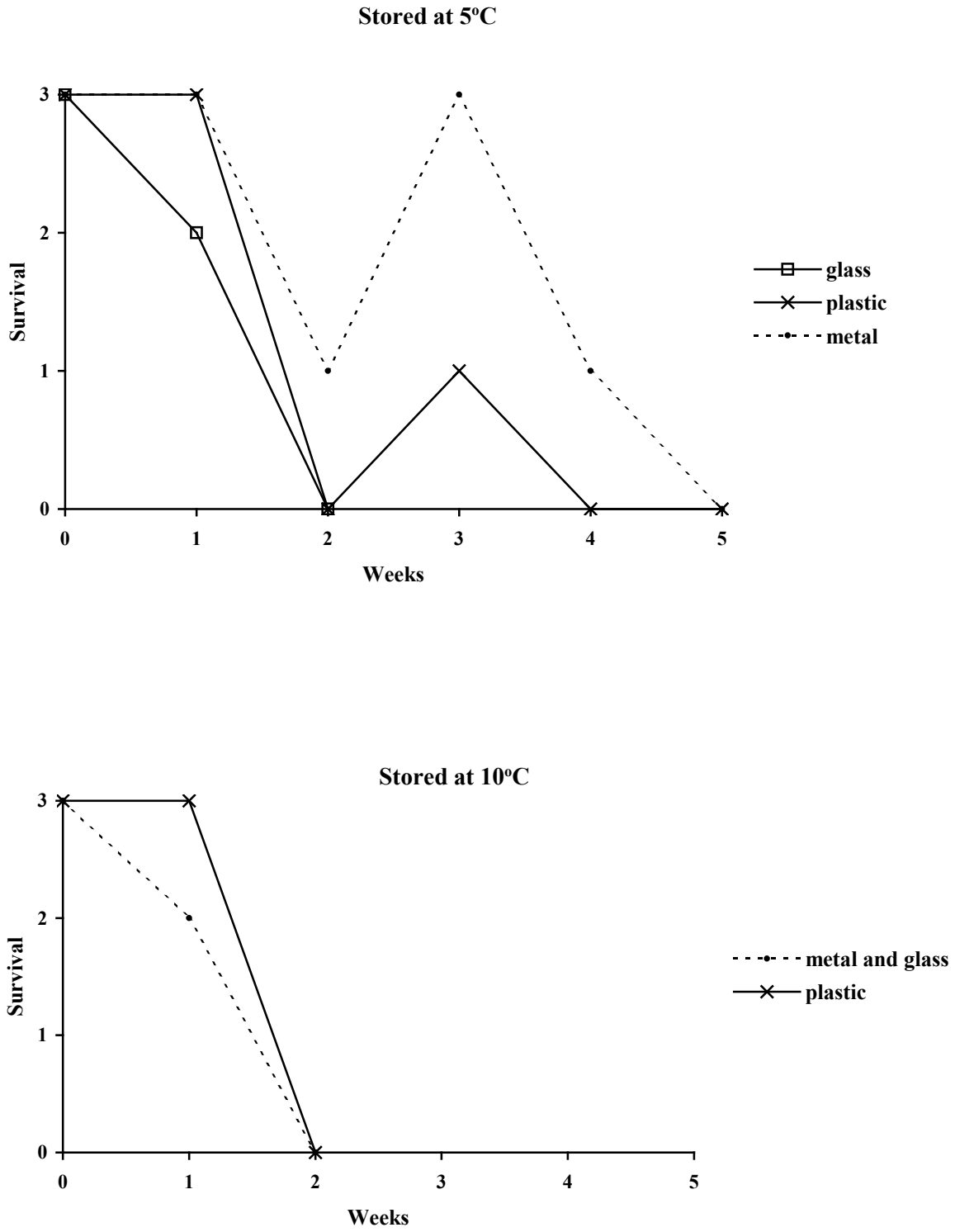
The results achieved at the two temperatures are shown in Table 4.1 and Figure 4.1.

Survival of PepMV was greater at 5 ºC than at 10 ºC. At 10 ºC, the virus survived for 1 week on all surfaces but not for 2 weeks. At 5 ºC, the virus survived for one week on glass, for 3 weeks on plastic and for 4 weeks on aluminium. No virus was detected on any surface after 5 weeks.

Table 4.1 Survival of PepMV in tomato leaf sap on 3 different surfaces at 5ºC and 10ºC at 80% RH

	No. of indicator plants (out of 3) positive for PepMV					
	5ºC			10ºC		
Time	Plastic	Metal	Glass	Plastic	Metal	Glass
2 days	3	3	3	3	3	3
7 days	3	3	2	3	2	3
14 days	0	1	0	0	0	0
21 days	1	3	0	0	0	0
28 days	0	1	0	0	0	0
35 days	0	0	0	0	0	0
42 days	0	0	0	0	0	0
49 days	0	0	0	0	0	0

Figure 4.1. Survival of PepMV on plastic, metal and glass at cool temperatures (5 and 10°C). Survival expressed as number of plants out of 3 plants.



Conclusions

1. PepMV at transmissible levels can occur widely on the glasshouse structure and equipment after an outbreak of the disease.

2. Survival of PepMV in dried sap is greater at cool temperatures (up to 4 weeks at 5°C) than at warm temperatures (4 days at 25°C).

3. PepMV occurs in tomato roots and can remain viable for at least 31 days in decaying roots in soil.

4. Nine disinfectants (Ben Glucid, Glucid, Horticide, Jet 5, Menno Florades, Panacide M, sodium hypochlorite, TSOP and Virkon S) are fully effective against PepMV in dried leaf sap when used at their recommended rates and with 1 hour contact time.

5. In a test with Horticide at the recommended rate, pepino mosaic virus was more difficult to decontaminate in fruit sap than in leaf sap. Horticide was not effective in the removal of PepMV from rigid plastic trays contaminated by squashed tomato fruit.

6. Virkon S and Unifect G are effective against PepMV in both dried leaf sap and fruit juice after just 1-minute contact time. Jet 5, Panacide M and sodium hypochlorite were not fully effective after 30 minutes.

7. Washing contaminated hands with Sensisept soap followed by Med gel eliminates transmissible PepMV. Washing in water alone is not effective.

8. Washing rigid plastic (e.g. picking trays) in water at more than 60°C for 3 minutes will remove contamination by PepMV in dried leaf sap and dried fruit sap.

9. Pressure washing rigid plastic with water at more than 50°C, and at 1300 psi, for 3 seconds, will remove contamination by PepMV in dried leaf sap and dried fruit sap.

Technology transfer (2000 – 2003)

1. Pepino mosaic virus of tomato- new results on virus persistence and disinfection. HDC Factsheet 19/03 (Tim O'Neill, Nicola Spence, Rick Mumford and Anna Skelton)
2. Wight Salads Grower Seminar, 25 July 2003 (Tim O'Neill)
3. Tomato Conference, 2 October 2003 (Nicola Spence and Tim O'Neill)
4. PepMV HortLINK proposal meeting, 13 August 2003 (Tim O'Neill)
5. Wright D, O'Neill TM, and Spence N (2002) Survival and disinfection of PepMV on surfaces. Proceedings international Virus Conference, Germany (poster)
6. New Results on Pepino mosaic virus. HDC Factsheet 11/01. (Tim O'Neill)
7. Pepino mosaic virus in tomatoes - an update on current research, Tomato Conference 2000 (Rick Mumford)
8. Progress in research on PepMV- Tomato Conference 2001, 12-14 (Nicola Spence, Tim O`Neill, Daphne Wright)
9. Pepino action plan. Grower, 7 December 2000, 22-23. (Tim O'Neill and Daphne Wright)

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