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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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PRACTICAL SECTION FOR GROWERS

Commercial benefit of the project

This project has identified potential sources of Pepino Mosaic Virus (PepMV) on affected nurseries and determined the likely survival period of the virus under different conditions. Nine disinfectants were shown to be capable of reducing virus contamination to non-transmissible levels. Application of the results will reduce the risk of continued outbreaks of PepMV on a nursery.

Background and objectives

Pepino mosaic virus was reported in the UK in a tomato crop in January 1999 and was subsequently confirmed in a further eight crops by September 2000. In the Netherlands the disease has been more widespread, with 52 outbreaks in 1999 and more than 25 by June 2000. It is a mechanically transmitted virus in the potex (*Potato virus X* (PVX)) group which appears to be highly contagious. Hands, clothing and tools are believed to be the primary means of spread. There appears to be a significant risk of carryover once a nursery is affected: 11 nurseries in Holland affected in 1999 were again reported to be affected early in 2000.

Infection in tomato results in a wide range of symptoms which commonly may include leaf mosaic and bubbling, spiky leaf margins, a pale green nettle-like head to the plant, angular bright yellow leaf spots and plant stunting; marbling and uneven ripening are common symptoms on fruit. Sometimes there are fruit symptoms but no leaf symptoms. Several varieties have been affected including both round and plum types. It is considered that the disease can cause substantial losses in protected tomato crops. MAFF-funded work has recently commenced to investigate various aspects of the detection and biology of PepMV. The objectives of the work described here are to provide practical information for growers on the major sources and survival of the virus on a nursery, together with recommendations of effective disinfectant treatments. As there are no chemical treatments to control the disease once plants are infected, hygiene is a key aspect for effective control of PepMV.

Summary of results and conclusions

Monitoring on two commercial nurseries revealed PepMV at transmissible levels on various surfaces and equipment in August 2000 when the disease was widespread in the crops. 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 affected for only 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. At the second nursery, no virus was detected on any of the surfaces tested following an end-of-season clean-up and disinfection with Horticide and Virkon S. However, PepMV was again detected in fruit debris found within one 'clean' house.

On a glass surface, PepMV survived in dried sap at transmissible levels for 2 but not 3 weeks at 15 ºC and 80% RH. At a warmer temperature (25ºC) the virus declined considerably within 48 hours and was not detected after 1 week.

Nine chemical disinfectants tested at their recommended rates 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, 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, particularly on rigid plastic. 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.

Recent findings on PepMV from MAFF-funded and overseas studies are summarised. Most of the common glasshouse weeds are non-hosts of PepMV; however, black nightshade and woody nightshade (bittersweet) are hosts, and could potentially act as reservoirs of the virus. PepMV has been confirmed in a wide range of tomato cultivars; no resistant cultivars have yet been identified. PepMV can occur naturally on tomato seed and, if seed are poorly cleaned, there is a risk that young tomato plants will become infected. No PepMV occurred when seed from infected fruit was acid-extracted, washed, dried, grown-on and 1500 resultant seedlings tested. The risk of transmission from infected tomato roots in the soil appears to be low, but plant to plant contact is a ready means of spread.

Action points for growers

<u>Persistence on a nursery</u>

1. Many surfaces in a glasshouse were readily 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, Appendix 1).*

2. While PepMV is relatively short-lived, persistence beyond 24 hours can be expected. *Movement of staff and equipment between houses risks spreading PepMV. Change to new coveralls, gloves and overshoes when moving between an infected and a 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 cooler, drier conditions - possibly beyond 2 weeks. *After an outbreak of PepMV, it is suggested that a glasshouse is maintained warm (e.g. 25^{o}C or greater) for 1 week between successive tomato crops.*

5. Although PepMV can occur in tomato roots in soil to at least 30 cm, the risk of transmission to new plants appears to be low. *Nevertheless*, i*t 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.*

Transmission from seed

6. 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, appears to be an effective way of eliminating this risk.*

Disinfection

7. 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 of disinfection on your nursery.*

8. 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. Robust disinfection methods for the removal of PepMV from rigid plastic trays contaminated by squashed tomato fruit, are not yet known.*

9. 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

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

Anticipated practical and financial benefits

As this disease is 'new' to Europe and to protected tomato crops, there is very little knowledge on how to control it. 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 between crops.
3) the effectiveness of chemical disinfection to limit spread and prevent carryover between successive crops.

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 and Wales. Losses result from:

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

It is estimated that losses in 1999 on the three UK affected nurseries were well in excess of £200,000.

SCIENCE SECTION

Introduction

Pepino mosaic virus has suddenly and seriously affected protected tomato production in the UK. 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 long-term the most effective method of control will be to breed resistant varieties (as with ToMV, where the Tm-2^2 gene has provided effective and durable control). In the short-term we need to identify the most effective precautions to limit spread and treatments to eradicate the virus.

The virus was first described in pepino (*Solanum muricatum)* in Peru in the 1970s during a survey of weeds to find natural hosts of potato virus diseases (Jones *et al.,* 1980). 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 20°C and for 6 months in desiccated *N. glutinosa* leaves. The virus was found to infect 30 out of 32 species of Solanaceae tested, all systemically. Significantly, tomato was found to be a symptomless host. It also infected *Cucumis sativus* (cucumber), though in inoculated leaves only (i.e. it did not become systemic). It failed to infect 13 species in 6 other families.

In Holland, a working group on PepMV was established to better understand the disease. 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°C under dry conditions for 1 day, not 4 days (survival under humid conditions is not known)
- disinfectants based on hydrogen peroxide did not work with a short contact time
- seed-borne infection is a possibility

This project is designed to:

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

1. Sources of PepMV in a glasshouse

Introduction

Pepino mosaic virus is highly contagious and very easily spread between plants by normal crop-handling practices. Virus particles within the sap are released when plants are handled. It is probable that equipment and surfaces within a glasshouse, as well as hands, will rapidly become contaminated by the virus. Monitoring was therefore undertaken in two glasshouses to determine which surfaces were contaminated and the relative frequency of contamination in different situations.

Materials and methods

Monitoring was undertaken on 8 August 2000 on two nurseries (Kent and Yorkshire), each of which contained a house where the virus had been present for several months and the crop was still in the house. At the Kent site, the glasshouse adjacent to the original infection had only recently become visibly infected and opportunity was taken to compare virus distribution in established and new outbreaks. The varieties were Eloise (new outbreak) and Santa (established outbreak) (Kent) and Santa (Yorkshire). 115 *Nicotiana benthamiana* indicator plants at the 6 leaf stage in 9 cm plant pots were taken to each site so that transmission tests could be performed *in situ*. Disposable gloves were used throughout and changed between each sample. Surfaces were swabbed using cotton buds soaked in phosphate buffer pH 7.0 containing a mild abrasive, celite. Each bud was stroked once across the test surface and then once on one leaf of an indicator plant. There were three replicate swabs per test location, each applied to a different leaf on the same indicator plant. The three cotton buds were then placed in a bottle of buffer solution which was subsequently tested for PepMV by ELISA. There were four replicate samples for each surface tested. Where possible, the leaf of a plant close to the swabbed surface was collected for testing. Indicator plants were placed in individual 'bread bags' to prevent contact between plants. Indicator plants were returned to CSL where they were grown-on in an aphid-proof glasshouse. Indicator plants were tested for PepMV by (i) examination for typical reactions on the indicator plants and (ii) by ELISA 2 weeks after inoculation.

Repeat monitoring was undertaken in November 2000 and December 2000 at the Kent and Yorkshire sites respectively, after the glasshouses had been cleared of infected crop, cleaned and disinfected.

Results and discussion

<u>Kent</u>
The test procedure worked well, with the results from negative and positive control plants as expected. PepMV at transmissible levels was confirmed on the main concrete pathway (surface positive; cracks negative); on picking trolleys (metal positive; canvas negative), waste containers (metal positive; canvas negative), irrigation lines, drip pegs, wooden support stakes at row ends and aluminium stanchions and aluminium CO_2 pipe at the glasshouse side (Table 1.1). The virus was also confirmed in volunteer tomato seedlings collected from next to the main pathway, though not from seedlings collected from outside. The virus was not

detected at transmissible levels on heating pipes or their supports, polythene floor covering, door knobs/chains, in run-off solution (central tank) or on disinfectant matting soaked in TSOP at the doorway.

PepMV was present at transmissible levels more frequently in the glasshouse with an established outbreak (11/56 samples) than in the recently affected glasshouse (3/48 samples).

The buffer solution in which the swabs were soaked revealed more widespread occurrence of PepMV than the indicator plant tests. These results suggest that the virus is either present at a very low concentration, or is incomplete (e.g. viral protein without DNA). The majority of tomato leaves collected from the crop with the established outbreak were positive for PepMV.

Yorkshire

PepMV at transmissible levels was confirmed on the polythene floor covering, picking trolleys, irrigation lines, drip pegs, run-off solution, glass walls and fork lift truck wheels (Table 1.2). The two areas of the glasshouse monitored had similar levels of PepMV contamination and this reflected similar levels of PepMV within the crop. The buffer solutions in which swabs were soaked revealed more widespread occurrence of PepMV than the indicator plant tests.

Over the two nurseries, PepMV was confirmed at transmissible levels on 11 of 15 surfaces tested. The exceptions were heating pipes, picking crates, door handles and disinfectant matting. On the heating pipes, picking crates and door handles, the virus was detected at non-transmissible levels.

Repeat testing after disinfection

Repeat testing of the Kent glasshouses was undertaken on 15 November 2000 using the procedures described earlier. The areas of the glasshouse which had previously tested positive were re-tested and some additional locations identified by the grower as difficult to clean. The glasshouses had been emptied of crops, cleaned and disinfected with TSOP. There was no crop in the house. No positive results were obtained on indicator plants suggesting an absence of PepMV at transmissible levels. However, PepMV was detected in some of the buffer solutions tested by ELISA (Table 1.3). In particular, PepMV was detected on picking crates and waste containers (not yet cleaned), on pipe stands, on concrete bases at the bottom of stanchions, on the main concrete pathway, on new polythene floor covering and debris trapped between the irrigation trickle line and nozzle. Tomato stem and fruit debris found within the house also tested positive, whereas volunteer tomato seedlings and fruit outside the house were all negative.

At the Yorkshire site, repeat testing of heavily infected glasshouses was undertaken on 19 December 2000 using the procedures previously described. One area of the glasshouse which had tested positive was re-tested but an area adjacent to the other infected area was being disinfected and no access was allowed. In this instance, surfaces outside the infected area in a block of cv. Tom Plum were sampled as before. As with the Kent glasshouses the areas had been emptied of crops, cleaned and thoroughly disinfected (washed down; fogged with Horticide; glass cleaned with GS4; misted with Virkon S immediately before planting). No PepMV was detected in

either the buffer solutions or the indicator plants tested by ELISA suggesting that the virus had been eradicated by the successful use of disinfectant. However, some fruit was still to be found on the floor under benches and in air-conditioning units which had apparently been missed in the clean-up procedure. These were tested and found positive both direct from the fruit and by inoculation to indicator plants.

Table 1.1 Occurrence of PepMV on equipment and surfaces in a glasshouse in Kent containing an infected crop - August 2000.

Equipment/surface tested	Nº of samples positive for PepMV (out of 4)					
	Established outbreak			New outbreak		
	A	B	C	A	B	C
1. Concrete path	1	4	4	0	0	0
2. Polythene floor covering	0	4	4	0	0	0
3. Run-off solution	0	0	-	0	0	-
4. Heating pipes	0	4	4	0	0	0
5. Picking trolleys	1	4	-	0	0	-
6. Picking crates	0	4	-	0	2	-
7. Waste containers	1	4	-	1	0	-
8. Irrigation lines	1	0	4	0	0	0
9. Drip pegs	3	1	4	2	0	0
10. Wooden support stakes	1	4	4	-	-	-
11. Door knob/chain	0	1	-	0	0	-
12. Glass side/Al pipe	0	0	0	2	1	-
13. Aluminium stanchion	3	4	4	0	0	1
14. Disinfectant matting	0	0	-	-	-	-

A Indicator plant results (PepMV at transmissible levels)
B Buffer solution results
C Adjacent tomato leaf
- Not tested

Table 1.2 Occurrence of PepMV on equipment and surfaces in a glasshouse in Yorkshire containing an infected crop - 8 August 2000.

Equipment/surface tested	Nº samples positive for PepMV (out of 4)[a]					
	Area 1			Area 2		
	A	B	C	A	B	C
1. Concrete pathway	0	1	2	0	0	1
2. Polythene floor covering	1	0	2(2)	0	1	1
3. Run-off solution	1	0	1(2)	0	0	2
4. Heating pipes	0	0	-	0	1	0
5. Picking trolleys	-	-	-	2	4	-
6. Picking crates	-	-	-	0	1	-
7. Waste containers	-	-	-	0	4	-
8. Irrigation lines	0	0	1(2)	1	0	1
9. Drip pegs	1	0	1	1	0	0(1)
10. Fork lift truck wheels	-	-	-	1	1	-
11. Door handles	-	-	-	0	0	-
12. Glass	-	-	-	1	0	-
13. Aluminium stanchions	0	0	1	0	0	1(2)

[a] Except where shown otherwise (in brackets)

A Indicator plant results (PepMV at transmissible levels)
B Buffer solution results
C Adjacent tomato leaf
- Not tested

Table 1.3 Detection of PepMV in two tomato glasshouses in Kent, 15 November 2000, after on outbreak of the disease in the previous crops.

Surface[a]	Number of samples testing positive for PepMV (total number tested)		
	Severe virus in previous crop	Slight virus in previous crop	Disinfectant used
Buffer solutions after swabbing			
1. Concrete path	**1(4)**	**-**	Trisop spray
2. Polythene floor covering	**1(4)**	**-**	New
3. Run-off solution	0(1)	-	Nothing
4. Heating pipes	0(2)	-	Trisop spray
5. Heating pipe stands	**2(2)**	**-**	Trisop spray
6. Picking trolleys	0(4)	-	Trisop spray
7. Picking crates	**4(4)**	**-**	Nothing
8. Waste containers	**1(4)**	**-**	Nothing
9. Irrigation lines	0(4)	0(4)	Trisop spray
10. Drip pegs	0(4)	0(4)	Trisop spray + wipe
11. Wooden stakes	0(2)	-	Nothing
12. Door knobs/chain	0(4)	-	Trisop spray
13. Aluminium pipe	0(4)	-	Trisop spray
14. Steel stanchions	0(4)	-	Trisop spray
15. Drip nozzles	**1(4)**	**-**	Trisop spray
16. Switches – cleaned	0(2)	-	Trisop swab
17. Switches - not cleaned	0(2)	-	Nothing
18. Concrete bottom of stanchion	**1(4)**	**-**	Trisop spray
19. Black waste pipe	-	-	Trisop dip
Plant samples			
1. Cherry tomato - inside	**-**	**Positive**	**-**
2. Stem debris - inside	**-**	**Positive**	**-**
3. Volunteer tomatoes outside- brown stem tissue	-	Negative	-
4. Volunteer tomatoes outside- green stem tissue	-	Negative	-
5. Volunteer tomatoes outside- squashed fruit	-	Negative	-

[a] Samples 1 - 19: Surfaces were swabbed with a cotton bud and then (i) sap transmission tests were made on indicator plants and (ii) the swabs were placed in buffer solution which was then tested by ELISA. No reactions were seen on any of the indicator plants (*N. benthamiana*) 12 days after inoculation and no PepMV was found in them when they were tested by ELISA.

Some positive results were obtained when the buffer solutions were tested by ELISA. These are shown above in bold.

2. Survival of PepMV

Introduction

Information on the period for which PepMV survives, on different surfaces and under different conditions, will help to provide guidelines on minimising carryover of the disease after an outbreak.

Materials and methods

Selected surfaces (polythene, concrete, cardboard, aluminium and glass) were contaminated with a natural inoculum by dipping the test surface into a solution (leaf sap diluted in phosphate buffer, 1 in 5) made from leaves of an infected tomato plant. The surfaces were maintained in a glasshouse, either exposed to daylight or in the dark, and four replicate swabs taken at regular intervals from 4 hours up to two weeks and transferred to indicator plants. The effects of temperature and humidity were assessed by contaminating glass slides with natural inoculum (as above), and placing them over saturated salt solutions in unilluminated incubators at 15, 20 and 25ºC in the dark. Swabs were taken and transferred to indicator plants until no further transmission occurred.

Results and discussion

Surfaces and daylight

At 21ºC and 71% RH, PepMV survived on all surfaces tested for at least 4 hours. It persisted on polythene, concrete and aluminium for 8 hours and on cardboard for 24 hours. There was a decline in recovery of PepMV with time and none was detected on any surface after 2 days. Daylight appeared to have little effect on survival of the virus in this test.

Table 2.1 Survival of PepMV on different surfaces in daylight and in the dark[a]

Sample interval	No. indicator plants (of 4) positive for PepMV				
	Polythene	Concrete	Glass	Aluminium	Cardboard
Daylight					
4 hours	4	0	3	2	2
8 hours	2	1	0	0	0
1 day	0	0	0	0	1
2 days	0	0	0	0	0
4 days	0	0	0	0	0
7 days	0	0	0	0	0
14 days	0	0	0	0	0
Dark					
4 hours	4	0	1	4	2
8 hours	4	0	0	2	1
1 day	0	0	0	0	0
2 days	0	0	0	0	0
4 days	0	0	0	0	0
7 days	0	0	0	0	0
14 days	0	0	0	0	0

[a] Average temperature was 21.3ºC; RH was 71%

Temperature and humidity

In an initial experiment, survival was greatest at the lower temperature (15ºC) and lower humidity (80%), with the virus still present at transmissible levels after 2 weeks under these conditions (Table 2.2). There was no reduction in virus transmission during the first 30 hours under any of the test conditions. Viability started to decline first at the highest temperature 25ºC and highest humidity 100% RH (Table 2.2).

Table 2.2 Survival of PepMV in tomato sap on glass at different humidities and temperatures in the dark.

Sample interval	Nº indicator plants (of 4) positive for PepMV			
	80%		100% RH	
	15ºC	25ºC	15ºC	25ºC
4 hours	4	4	4	4
8 hours	4	4	4	4
12 hours	4	4	4	4
24 hours	4	4	4	4
30 hours	4	4	4	4
48 hours	4	2	4	1
4 days	4	1	1	0
1 week	4	0	1	0
2 weeks	3	0	0	0

In a second experiment, the effect of temperature on survival of PepMV was investigated at a constant RH (80%). The virus survived for 2 weeks at 15ºC, 4 days at 20ºC and 2 days at 25ºC (Table 2.3). No virus was detected at 3 weeks.

In a third experiment, the effect of relative humidity on survival of PepMV at a constant temperature (20ºC) was investigated (Table 2.4). The virus survived for 2 days at 60% RH and for 1 week at 80% and 100% RH. The slight effect of RH was in the opposite direction to that found in experiment 1.

In summary, survival of PepMV in tomato sap on glass is significantly influenced by temperature while the effect of humidity is not clear cut. Survival was least at the high temperature (2 days but not 4 days at 25ºC) and was greatest (2 weeks, but not 3 weeks) at a lower temperature (15ºC).

Survival in tomato leaf debris may be greater than 2 weeks. The original Peruvian isolate of PepMV was shown to survive for at least 6 months in *N. glutinosa* leaves dried over silica gel (Jones *et al.,* 1980).

Table 2.3 Survival of PepMV in tomato sap on glass at three temperatures at 80% RH

Sample interval	No. indicator plants (of 4) positive for PepMV		
	15ºC	20ºC	25ºC
1 day	4	4	4
2 days	4	4	3
4 days	4	3	0
1 week	4	0	0
2 weeks	2	0	0
3 weeks	0	0	0
4 weeks	0	0	0
5 weeks	0	0	0

Table 2.4 Survival of PepMV in tomato sap on glass at three humidities at 20ºC

Sample interval	No. indicator plants (of 4) positive for PepMV		
	60% RH	80% RH	100% RH
1 day	4	4	4
2 days	4	4	2
4 days	-	-	-
1 week	0	2	2
2 weeks	0	0	0
3 weeks	0	0	0
4 weeks	0	0	0

- = not determined

3. Survival of PepMV in soil

Introduction

At least two of the UK outbreaks of PepMV have been in soil-grown crops. With *Tomato mosaic virus* (ToMV), it is well known that the virus can overwinter in root debris in the soil, which can act as a source of infection for the next crop (Fletcher, 1969; Lanter 1982; Pares *et al*., 1996). Survival of ToMV is greater in thick root pieces and in uncultivated soil. Dutch studies indicate that high concentrations of PepMV occur in tomato roots. Information is urgently needed on the risk of carryover of PepMV in tomato root debris in the soil. The following is a summary of tests on root samples arranged by one UK grower following an outbreak on his nursery in a soil-grown crop.

Materials and methods

Soil grown tomato plants with obvious symptoms of PepMV in a naturally infected crop were selected and their position marked. Pieces of root were collected at intervals and sent to CSL for testing by ELISA and by sap inoculation onto indicator plants.

Experiment 1
A soil grown tomato crop with obvious symptoms of PepMV was cut-off at ground level and all large roots were removed from the soil. The soil was then rotovated to reduce the size and enhance the decay of fine root pieces remaining in the soil. The soil was sifted and tomato roots were recovered from 10 positions, at approximately 8 weeks after crop removal, and tested for PepMV.

Experiment 2
Two plants showing obvious symptoms of PepMV in a crop of cv. Espero were cut off at the stem base on 19 September. Three root samples (at soil level, 0-15 and 15-30 cm depth) were taken from each of the two plants and tested for PepMV.

Experiment 3
Root samples from 15 cm depth were collected from four groups of three plants. The central plant of each group showed obvious symptoms of PepMV while the plants either side did not show symptoms. Samples were collected at intervals from 20 September to 15 November. All of the plants were removed on 19 September. Roots were taken from close to the main root to minimise risk of inadvertently sampling the roots of an adjacent plant.

Results

Experiment 1
No PepMV was detected in fine root pieces recovered approximately 8 weeks after removal of the infected tomato crop.

Experiment 2
PepMV was detected in root pieces sampled from 0, 15 and 30 cm depth at the time of removal of infected tomato plants.

Experiment 3
PepMV was not found at transmissible levels in the roots of obviously affected plants, at 17 days after plant removal or subsequently. PepMV was however, detected in root pieces from apparently healthy (but presumably infected) plants adjacent to the original infected plants. The virus was detected at 31 days after removal of the tops, but not at 57 days.

Table 3.1 Persistence of PepMV in tomato roots in soil.

Date of root sampling[a] (CSL ref nº.)	Days from plant removal	Nº samples testing positive for PepMV		
		Nº plants sampled	Direct test on roots (ELISA)	Transmission to indicator plant[b]
Plant with symptoms				
1. 19 September (6201-)	0	-	-	-
2. 6 October (6327-)	17	4	4	0
3. 20 October (6651-)	31	4	1	0
4. 15 November (7211-)	57	4	1	0
Adjacent plants, no symptoms				
1. 19 September (6201-)	0	3	1	0
2. 6 October (6327-)	17	8	4	4
3. 20 October (6651-)	31	8	5	2
4. 15 November (7211-)	57	8	3	0

[a] Plants were cut off at soil level on 19 September.
[b] Symptoms on indicator plant and ELISA positive.

Discussion

These tests confirm that PepMV can occur in tomato roots at depths at least to 30 cm in soil. The virus was not detected at transmissible levels in roots 57 days after plants were cut-off at soil level, indicating in this experiment a survival period of less than 8 weeks in decaying roots. Other experiments indicate the risk of transmission from soil, via infected roots, is low - see section 5.

4. Comparison of disinfectants

Introduction

There is no independent published information on the effectiveness of different chemical disinfectants against PepMV. Previous studies on other mechanically transmitted virus diseases (e.g. *Tomato mosaic virus, Pepper mild mosaic virus* and C*ucumber mosaic virus*) suggest that TSOP and Virkon S are likely to have some effect on PepMV (Stijger 1993; Broadbent, 1976). Product literature reports that Menno-Florades is effective against PepMV. It would be useful to know if products currently used to disinfect glasshouses after tomato crop production (e.g. Jet 5, GluCid, Horticide, Sodium hypochlorite) and previously shown to be effective against other important tomato diseases, are also effective against PepMV.

Materials and methods

Virus isolate. The isolate of PepMV was supplied by CSL as infected freeze-dried *Nicotiana benthamiana* leaves. The virus was maintained in *N. benthamiana* throughout.

Production of inoculum. Leaves of tomato cv. Alicante were dusted with 600-mesh carborundum and using muslin inoculated manually with infective *N. benthamiana* sap in inoculation buffer. Inoculated plants were grown at 15-18°C and tested for infection by electron microscopy examination using the "quick dip" method.

Disinfectants. For each disinfectant a series of four concentrations were made using the recommended rate provided by suppliers and dilutions with distilled water down to 1/8 of the recommended rate. Disinfectants tested are shown in Table 4.1.

Inoculation of surfaces.
Infected tomato leaves were homogenised using a pestle and mortar. This material was then filtered through a layer of muslin and the infective sap was rubbed onto each of the five surfaces: aluminium, concrete, glass, polythene and rigid plastic (polypropylene). The surfaces were then sprayed at an application rate recommended by the supplier. A set of surfaces were also sprayed with distilled water (water spray control) and not sprayed at all (no spray control). In the final experiment infected tomato fruit were homogenised using a pestle and mortar. This material was then filtered through a layer of muslin and the infective tomato juice was rubbed onto each of the five surfaces as before.

The plants were then misted with distilled water. The infectivity test was conducted by rubbing a small muslin square over the sprayed surface, after the correct exposure time, and subsequently rubbing three leaves of each *N. benthamiana* plant. There were three plants per exposure time.

Assessment of infection. The development of symptoms in *N. benthamiana* was monitored for three weeks. Initially plants were scored after 4-6 days, when the first symptom, a distinct yellowish mosaic in leaf tips, was apparent. Plants were then scored again at regular intervals, where older leaves develop a generalised milder mosaic and/or inoculated and lower uninoculated leaves develop irregular expanding

necrotic patches.

Three weeks after inoculation all plants were sampled and replicates were bulked together. These bulked samples were then tested for the presence of PepMV by DAS-ELISA. The plates were coated with a purified IgG used at 1mg ml^{-1} and IgG conjugated to alkaline phosphatase used at 1/1000, supplied by CSL.

Table 4.1 Details of chemical disinfectants used

Product/commodity material	Active ingredients	Rates of products used (recommended rate and dilutions)			
		x 1	x $^1/_2$	x $^1/_4$	x $^1/_8$
1. Benglucid	5-10% benzoalkonium chloride + 23% glutaraldehyde	2%	1%	0.5%	0.25%
2. Glu-Cid	20% glutaraldehyde	2%	1%	0.5%	0.25%
3. Horticide	15% glutaraldehyde + 10% QAC	1:25	1:50	1:100	1:200
4. Jet 5	hydrogen peroxide + peracetic acid	1:125	1:250	1:500	1:1000
5. Menno-Florades	9% benzoic acid + propanol	4%	2%	1%	0.5%
6. Panacide M	30% dichlorophen + 9% sodium hydroxide	0.5%	0.25%	0.125%	0.0625%
7. Sodium hypochlorite	10-14% available chlorine	400	200	100	50 ppm
8. TSOP	trisodium orthophosphate	10%	5%	2.5%	1.25%
9. Virkon S	organic acids and salts	1%	0.5%	0.25%	0.125%

Results and discussion

Ben-Glucid was 100% effective at disinfecting all surfaces at the recommended rate and at a dilution of half the recommended rate after 1 hour. On rigid plastic it was effective at all dilutions. However, its performance at higher dilutions was better on aluminium, glass and rigid plastic than on concrete and polythene. After 4 hours it was 100% effective on all surfaces at every dilution.

Menno-Florades was 100% effective at disinfecting aluminium, glass, polythene and rigid plastic at the recommended rate after 1 hour. However, it performed less well on concrete and PepMV was detected after 2 hours.. After 6 hours it was 100% effective on all surfaces at every dilution tested.

Virkon S was 100% effective at disinfecting all surfaces at the recommended rate and disinfected glass at all dilutions. After 1 hour it was 100% effective for both the recommended and half recommended dilutions. After 4 hours it was 100% effective on all surfaces at every dilution.

Glucid was 100% effective at disinfecting all surfaces at the recommended rate and in disinfecting aluminium and rigid plastic at all dilutions. After 1 hour it was 100% effective for the recommended rate and at dilutions of 1/2 and 1/4. After 2 hours it was 100% effective on all surfaces at every dilution.

Jet 5 was 100% effective at disinfecting all surfaces at the recommended rate and disinfecting concrete and glass at all dilutions.

TSOP was 100% effective at disinfecting all surfaces at the recommended rate and disinfecting concrete, glass and plastic at all dilutions.

Horticide was 100% effective at disinfecting all surfaces at the recommended rate and at all dilutions. This was the only disinfectant that did this. When Horticide was applied to surfaces contaminated with PepMV from infected tomato fruit it was 100% effective at disinfecting all surfaces, except rigid plastic at the recommended rate. On aluminium, concrete, glass and polythene is was effective at 50% of the recommended rate after 1 hour.

Panacide M was 100% effective at disinfecting all surfaces at the recommended rate and disinfecting plastic at all dilutions.

Sodium hypochlorite was 100% effective at disinfecting aluminium, glass and rigid plastic at the recommended rate, but not glass or polythene. It disinfected only concrete at all dilutions.

Water spray control - PepMV in tomato leaf

Interestingly, the distilled water spray also had an effect. In each experiment the virus was inactive at 1 hour on concrete and water sprayed on to some of the other surfaces also appeared to reduce contamination. This action may simply be due to washing the viral inoculum off the surfaces. Alternatively it may in some part be due to changes in humidity. However, the results are variable, so water alone should not be considered as a sufficient treatment. Most importantly, the virus appears to remain viable for longer on polythene and rigid plastic than on other surfaces.

Water spray control - PepMV in tomato fruit

Distilled water spray had almost no effect when sprayed on surfaces contaminated with PepMV from tomato fruit. This was in contrast to contamination by infected leaf material.

No spray control

A control carried out where swabs were taken from contaminated surfaces which were not sprayed at intervals up to 24 hours. This resulted in 100% PepMV infection.

Table 4.2 Effect of various disinfectants at different dilutions on the infectivity of PepMV on glasshouse surfaces over time.[a]

Disinfectant	Time (hrs)	Dilution of recommended rate of disinfectant used on different glasshouse surfaces																			
		Aluminium				Concrete				Glass				Polythene				Plastic			
		1	1/2	1/4	1/8	1	1/2	1/4	1/8	1	1/2	1/4	1/8	1	1/2	1/4	1/8	1	1/2	1/4	1/8
Ben-Glucid	1	-	-	+	-	-	-	+	+	-	-	-	+	-	-	-	+	-	-	-	-
	2	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Menno-Florades	1	-	-	+	-	-	+	+	-	-	+	-	-	-	-	-	+	-	-	+	+
	2	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glucid	1	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Virkon S	1	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+
	2	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	+
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Jet 5	1	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
	6	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TSOP	1	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Horticide	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(leaf sap)	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Horticide	1	-	-	+	+	-	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+
(fruit sap)	2	-	-	-	+	-	-	+	+	-	-	-	+	-	-	+	+	-	-	+	+
	4	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+
	6	-	-	+	+	-	-	+	+	-	-	-	+	-	-	-	+	+	+	-	-
	24	-	-	+	-	-	-	-	+	-	-	-	+	-	-	+	+	+	-	-	+
Panacide M	1	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-
	2	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-
	24	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-

Table 4.2 continued

Sodium hypochlorite	1	- - - +	- - - -	- - - -	- - - +	- - - +
	2	- - - -	- - - -	+ - - +	+ - - +	- - + +
	4	- - - -	- - - -	- - - -	+ - - -	- - - -
	6	- - - -	- - - -	- - - -	+ - - -	- - - -
	24	- - - -	- - - -	- - - -	- - - -	- - - -
Water spray control	Time (hrs)	Aluminuim	Concrete	Glass	Polythene	Plastic
		Experiment	Experiment	Experiment	Experiment	Experiment
		1 2 3	1 2 3	1 2 3	1 2 3	1 2 3
	1	- - +	- - -	+ - +	+ + +	+ + -
	2	- + -	- - -	- - +	+ + +	- + +
	4	- - -	- - -	+ - -	- - +	- + +
	6	- - +	- - -	- - -	- + -	- + -
	24	- - -	- - -	- - -	- + -	- - -
Water spray control: infected tomato fruit		Aluminuim	Concrete	Glass	Polythene	Plastic
	1	+	+	+	+	+
	2	+	_	+	+	+
	4	+	+	+	+	+
	6	+	+	+	_	+
	24	+	+	+	+	+
No spray control	Time	Experiment	Experiment	Experiment	Experiment	Experiment
		1 2 3	1 2 3	1 2 3	1 2 3	1 2 3
	1	+ + +	+ + +	+ + +	+ + +	+ + +
	2	+ + +	+ + +	+ + +	+ + +	+ + +
	4	+ + +	+ + +	+ + +	+ + +	+ + +
	6	+ + +	+ + +	+ + +	+ + +	+ + +
	24	+ + +	+ + +	+ + +	+ + +	+ + +

+ infective PepMV detected; - no pepMV detected.

[a] Surfaces were contaminated with tomato leaf sap containing PepMV; Horticide was also tested against infested fruit sap.

Conclusions

All products tested were fully effective in decontaminating all surfaces of PepMV in leaf sap after 1 hour when used at the recommended rate, except Menno-Florades on concrete and sodium hypochlorite on glass and polythene. Dilution of the manufacturer's recommended rate resulted in variable efficacy, however all products tested were 100% effective in decontaminating all surfaces after 24 hours at dilutions down to one eighth of recommended rates. Horticide was the only disinfectant that was 100% effective at disinfecting all surfaces at the recommended rate and at all dilutions. In general, all disinfectants performed well, and even water alone had some effect on some surfaces. This indicates that the viability of PepMV on glasshouse surfaces is not good if surfaces are sprayed; this may in part be due to changes in humidity. PepMV was more difficult to decontaminate in fruit sap than in leaf sap.

5. Summary of research on PepMV

Literature searches were undertaken in June 2000 and April 2001, and the results of MAFF-funded work on PepMV were made available to the project leader. Key points are summarised below by topic.

Symptoms in tomato

Symptoms are illustrated in HDC Factsheet 12/00 and in various articles in HDC News, Grower and Commercial Greenhouse Grower (see reference list). Symptoms are extremely variable and often only one or a few of the following are seen:

- Nettlehead' symptom (leaves in the head are held very upright; leaves are crinkled and distorted)
- Bright yellow leaf spotting and /or mosaic
- Leaf bubbling and distortion; spiky leaf margin (fewer indentations)
- Narrowed leaf tips
- Darker green lower leaves
- Necrotic leaf spotting. First symptoms can be small, necrotic spots (1-2 mm diameter) on older leaves. They do not increase in size and can be easily overlooked
- Interveinal chlorosis (cf. iron deficiency)
- Hollow stems
- Wilting
- Stunted growth
- Yellow/orange rings and blotches on fruit, which fail to ripen; marbled appearance. The fruit may have a red band running from the calyx end to the opposite end.
- Pitting (dimpling) of the fruit surface
- Reduced fruit yield. First fruiting delayed.

Effect of growing environment on symptoms

Symptoms are reported to be more apparent during periods of stress, including low light and generative growth. Symptoms appear 10 - 14 days after artificial inoculation, but in practice this period may be longer. An incubation period of up to 12 weeks between infection and symptom development was reported in Holland (Cooke, 1999).

The effect of the environment on symptom expression is not well understood. It is thought that in conditions of low light, and possibly slightly lower temperatures, the leaflets show distortion and the nettlehead symptom, but in high light conditions and at higher temperatures leaf yellowing symptoms are more apparent. Infected mature plants can also show few or no leaf symptoms, but very obvious fruit symptoms.

Effect on tomato yield

In a small glasshouse experiment conducted at CSL on cv. Espero grown on rockwool slabs, with plants inoculated at 6 weeks old:

- Total yield reduced by 15.4% (over the period weeks 12 to 26).
- Reduction in both fruit number (5.5%) and fruit size (9.2%).
- First pick delayed by 2 weeks.
- 10% drop in Class I fruit.
- Marked stunting in growth was evident 5 weeks after inoculation.

Host range

MAFF-funded studies at CSL indicate that PepMV is restricted to Solanaceous hosts, with the exception of cucumber where local (not systemic) infection was observed following inoculation.

Natural outbreaks of PepMV in these plants
Pepino (*Solanum muricatum)*
Tomato (cvs. Eloise, Espero, Santa, Solairo, Aranca, Nectar, Golden Harvest, Rosafino, Candella, Sweet Lady)

Plants infected on inoculation (i.e. symptoms produced)
Potato *(Solanum tuberosum)*
Aubergine *(Solanum melongena)*
Tobacco *(Nicotiana tabacum)*
Black nightshade *(Solanum nigrum)*
Woody nightshade *(Solanum dulcamara)*

Of 25 potato varieties inoculated with a UK isolate of PepMV, 20 were found to be susceptible and 5 appeared to be resistant (negative on testing by ELISA after inoculation). Some of the susceptible varieties showed no symptoms, others showed necrotic lesions on leaves and in susceptible varieties there was efficient transmission of the virus to progeny tubers.

Plants not infected on inoculation (i.e. non-hosts)
Edible
Pepper (pepper can therefore be used as a break crop after an outbreak)

Weeds
Chickweed *(Stellaria media)*
Curled dock *(Rumex crispus)*
Dandelion *(Taraxacum officinale)*
Groundsel *(Senecio vulgaris)*
Hairy bittercress *(Cardamine hirsuta)*
Nettle *(Urtica dioica)*
Shepherd's Purse *(Capsella bursa-pastoris)*

Ornamentals
Argyanthemum
Dahlietta
Fuchsia
Nicotiana alata
Pelargonium
Petunia
Petunia (trailing)

Seed transmission

PepMV has occasionally been detected on tomato seed, at very low incidence, but plants which germinated from contaminated seed were free from infection. In experimental studies, acid-extracted washed and dried seed from PepMV-infected fruit was grown on and 1500 seedlings tested. No PepMV was detected when leaves were tested after 4 weeks. However, in recent experiments in The Netherlands, using tomato seed collected from PepMV infected plants, and in which the seeds were partly cleaned by natural fermentation and drying (rather than acid extraction, as is normal) the virus was confirmed at a very low level in resultant seedlings. PepMV was found in 3 of 5,200 seedlings (0.06%) and in 2 of 8,200 seedling (0.03%). In further tests it was shown that the virus was present on the outside of seeds, and not in the embryo or endosperm. The level of virus decreased with seed storage though it could still be detected at infectious levels (by inoculation test onto assay plants) after 8 weeks. Acid extraction and disinfection treatments (trisodium phosphate and sodium hypochlorite) effectively reduced the amount of virus detectable, confirming its external localisation on seed.

Seed testing

It is reported that if ELISA is used for seed testing, a sample size comparable to that used for tobamoviruses (3,000 seeds) would be more than sufficient (Krinkels, 2001). When a single PepMV infested seed was added to 250 non-infested seed to create a 'spiked' batch (0.4% seed infested) all of six testing laboratories in The Netherlands were able to identify the spiked batches.

Transmission from soil

The possibility of PepMV being transmitted via infected root pieces in the soil appears to be low. When infected tomato roots (1-2 cm in length) were incorporated into potting compost, and the pots planted with 2 week old tomato seedlings, no virus was detected in the bait tomato plants 7 weeks later.

Testing of young plants

The Dutch Inspection Service for Horticulture (Naktuinbouw) has tested over 350 batches of young plants, by ELISA testing of leaf samples, and did not find PepMV in any of them.

Tobacco as a source of PepMV?

Hand-rolled tobacco was tested at CSL, but the only virus found was *Tobacco mosaic virus* (TMV).

Spread by bees?

Observation on UK nurseries where infected plants have tended to occur as groups of adjacent plants along a row rather than as individual plants scattered through the house, suggest that human activity, rather than bees, is the main method of spread. In a trial in Holland, where a high number of bees were used in a tomato crop, the bees

did not appear to be an important vector of the virus.

Countries where PepMV has been confirmed in tomato

Austria	Italy	Ukraine
Belgium	Morocco	UK
Canada	The Netherlands	USA
France	Portugal	
Germany	Spain and the Canary Isles	

Strain identification

The UK isolates of PepMV from tomato have been shown to be very similar to European isolates from tomato, with over 99% of their genomic RNA identical. These isolates from tomato appear to be a distinct strain from the original PepMV isolates from pepino in Peru, differing in RNA sequence by 4-5%. The original Peruvian isolate of PepMV infects tomato but does not induce symptoms.

Quarantine status

PepMV was given temporary quarantine status by an EC Commission Decision in 2000, authorising Member States provisionally to take measures against the virus and its introduction into and spread within the Community. This was subsequently extended to 31 December 2002 and, in view of the probability that seed plays an important role in the spread of PepMV, extended to apply to tomato seed as well as tomato plants and fruit.

Detection methods

In MAFF-funded work, CSL have developed a method for rapid diagnosis of the virus by growers, crop consultants and Plant Health and Seeds Inspectors, using a 'lateral-flow' kit. The device works with both leaf tissue and fruit skin samples and gives a clear result in a few minutes. There is no need to keep the kits refrigerated, and an internal control line is included to check that the test has worked. The kit was shown to detect all PepMV isolates tested and it did not cross-react with *Tomato Mosaic Virus* (ToMV), *Potato Virus X* (PVX) or *Tomato Spotted Wilt Virus* (TSWV). The kit is available to growers, from CSL, in the Pocket Diagnostic range of test kits.

Polyclonal antibodies to PepMV are available commercially and monoclonal antibodies have been raised by CSL. One has been selected by CSL for use in routine ELISA assays for detection of PepMV.

A TaqMan assay for PepMV has been developed by CSL and shown to be extremely sensitive, capable of detecting the virus down to one in a million dilution.

Disinfection tests

A trial at Humboldt University, Berlin, demonstrated Menno-Florades was effective against PepMV. It was recommended that the product was used as follows: 3% for 3 minutes to disinfect knives and equipment; 2% for 16 hours to disinfect hard surfaces. When dipping knives and equipment, it is recommended that the pH of the dipping solution is kept below 4.5; monitor with pH paper and add more Menno Florades as necessary

Product literature for Vitax Vitafect reports this disinfectant was found to be effective against PepMV in trials with the sap of infected plants. For use against PepMV the product is recommended at 2% v/v, leaving for 30 minutes before rinsing surfaces with clean water.

Research needs

1. Survival of PepMV in dried tomato tissue (e.g. leaf debris; fruit pulp) and fruit sap.
2. Robust disinfection methods for the decontamination of rigid plastic trays infected with PepMV from squashed fruit/fruit sap.
3. Survival in soil at low levels; use of bait tests.
4. Effect of factors on symptom expression (isolates, variety, age of plant when infected, temperature, day length etc).
5. Efficacy of disinfectants when used as a 'quick dip' (e.g. for knives).
6. Possibility of cross-protection using a mild strain.
7. Disinfection of recycled nutrient solution; transmission through sand filters.

Conclusions

1. In glasshouses containing infected tomato crops, PepMV was detected at transmissible levels on 11 out of 15 surfaces tested. It was most common on drip pegs, picking trolleys and glass walls. It was found in the nutrient solution on one nursery using NFT production.

2. On three out of the four remaining surfaces (heating pipes, picking crates and door handles), the virus was detected at non-transmissible levels. It was not detected at all on matting soaked in disinfectant (TSOP) used as a foot and wheel dip.

3. Contamination of surfaces was more common in a glasshouse where the crop had been affected for several months, than where the crop was recently infected.

4. PepMV was not found on surfaces at transmissible levels in the test glasshouses in mid-November 2000 and mid-December 2000, after cleaning and disinfection with TSOP. However, the virus was found in fallen fruit overlooked during the nursery clean-up.

5. PepMV persisted at transmissible levels for 2 weeks but not 3 weeks on glass, in the dark at 15ºC. Persistence was least at 25ºC where the virus started to decline after 2 days and was not detected at 4 days.

6. PepMV was detected in tomato roots at 30 cm depth in the soil.

7. Nine disinfectant products tested at their standard rate were effective at preventing transmission of PepMV.

8. PepMV is more difficult to decontaminate in fruit sap than in leaf sap.

9. Research on PepMV at CSL (MAFF-funded), and published European information, is summarised.

Technology Transfer

1. Presentation at the UK Tomato Conference, Coventry, 28 September, 2000 (Rick Mumford and Nicola Spence).
2. O'Neill T M and Wright D (2000). Pepino action plan. *Grower*, 7 December, 22-23. [Appendix 1]
3. Grower seminar organised by Mr P Morley, Isle of Wight, 9 October 2000 (Tim O'Neill).
4. Hortex Technical Seminar, Telford, 16 January 2001 (Rick Mumford).

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Appendix 1 - [O'Neill T M and Wright D (2000). Pepino action plan. *Grower*, 7 December, 22-23]

Pepino action plan

Tim O'Neill of ADAS Arthur Rickwood and Daphne Wright of CSL York provide guidance on how to check the new season's tomato crop for *Pepino mosaic virus* (PepMV), together with precautions on minimising the risk of unknowingly spreading this highly contagious disease.

Background

PepMV was first recorded in a UK tomato crop in January 1999 and was followed by a second outbreak later that year. In the 1999/2000 growing season there were seven cases, two of which were eradicated by very prompt action. This mechanically transmitted virus disease can spread extremely rapidly through a crop, significantly reducing marketable yield and grade. Originally found in pepino plants (*Solanum muricatum*) in Peru in 1980, the virus has more recently been identified in tomato crops in the Netherlands, Spain, France, Belgium, Germany, Austria and the USA. The disease also poses a potential threat to potato and aubergine crops.

No consistent source of the problem has been identified so far. Various tomato varieties and types from different seed houses have been affected. The possibility of smoking tobacco harbouring the virus was considered (it has previously been shown to be a source of ToMV) but samples tested to date have all proved negative. The most likely sources are: contaminated seed; handling infected plants or fruit; carryover in tomato crop debris from a previous outbreak; carryover on trays, boxes or other equipment brought onto a nursery, from within the UK or overseas.

Symptoms are extremely variable, making accurate diagnosis difficult, especially early in the season. However, an on-site rapid diagnostic kit is now available and will enable suspect symptoms to be checked rapidly. The diagnostic kit costs £14 + VAT for a box of four devices, available from CSL York (email: pocketdiagnostics@csl.gov.uk or telephone 01904 462600). Confirmatory testing by an ELISA test at CSL takes around 2-3 days from receipt of samples.

PepMV is a notifiable disease. If you suspect the problem, you must notify the Plant Health and Seeds Inspectorate (PHSI). Where it can be identified early on a nursery, the infected plants and those around it are taken out. In more serious cases, the policy is one of containment by strict hygiene and working restrictions to slow its spread.

Inspecting the new crop

Inspect plants on arrival, and regularly during the first few weeks of growth. Symptoms of PepMV can occur as early as December or January. Do not handle plants whilst walking along the row to check them! Prompt and careful removal of all infected plants and establishment of a cordon sanitaire (a safety barrier of cleared healthy plants) has proved successful in controlling the disease on some nurseries.

Symptoms to check for are leaf bubbling and distortion (Figures 1 and 2) and a 'nettlehead' symptom in which the leaves are held more rigid and upright than usual and have a pale yellow mosaic (Figure 3). Pay particular attention to the plant heads and scan them in the rows 1 or 2 across from the pathway you are walking in. The odd plant or group of plants can be picked-out more easily this way. Later in the season a bright yellow leaf mosaic may occur (Figure 4). Plants may also show a reduced growth rate, or simply stop growing in the head and become stunted. Usually only one or two of these symptom types are present in an infected crop. The particular symptoms occurring are thought to be determined by a range of factors including variety, plant age, time of year (e.g. light level) and age when a plant is infected.

As the virus is very easily spread by handling plants and by clothing, staff working in the crop are the most common method of rapid secondary spread. Groups of infected plants can often be found, with the outbreak spreading in the direction of crop working. The initial outbreak may be restricted to an area of crop managed by one person.

Action on finding a suspect infected plant

- Inform your local Plant Health and Seeds Inspector that you suspect PepMV. See under Ministry of Agriculture, Fisheries and Food in the telephone directory; or telephone PHSI headquarters in York (01904 455174).

- Mark the string of the suspect plant/s with coloured tape or other obvious marker. Suspend all work in this area until the test for PepMV has been completed.

- Physically isolate the suspect area and surrounding rows to prevent anyone going into the area inadvertently. Disinfect all trolleys, boxes, knives and other equipment which has been used in this area.

- Take a sample by placing your hand inside a polythene bag and picking off the leaves showing symptoms (using the bag as a glove), turn the bag inside out drawing the sample leaves into it, thus avoiding contamination of the outside of the bag.

- The sample can be tested on site using the diagnostic kit.

- Normally your local PHSI will visit and arrange for the sample to be sent to CSL for testing; if this is not possible, arrangements can be made for samples to be sent directly to CSL.

- If PepMV is confirmed, carefully remove the plants into polythene bags, and a surrounding zone of at least 3 slabs or 12 plants either side. If the crop is not on the V-system, also remove plants in the adjacent row. Dispose of plants as instructed by PHSI. If there are several affected plants scattered along a row, remove the whole row. Consider erecting a temporary polythene screen if a large number of plants are to be removed from the crop (Figure 5). Place a notice on the entrance door alerting staff and visitors that PepMV is present in the glasshouse and extra hygiene precautions apply.

General precautions

- In HDC - funded work this autumn (Project PC 181) we readily detected PepMV at transmissible levels in glasshouses containing infected crops. Surfaces where we commonly detected the virus included aluminium stanchions, concrete pathways, drip pegs and irrigation lines, wooden stakes at row ends, trolley wheels and waste containers.

- Make sure that the glasshouse and all equipment used in it (e.g. boxes, trays, forklift trucks) has been thoroughly cleaned and disinfected before the new crop arrives. Remove all traces of the previous crop. Do not shred the haulm when removing it.

- Leave the glasshouse empty for as long as possible between crops, at least 3 weeks, and ensure that the temperature is no less than 20ºC during this period. In laboratory tests we found that the virus survived on surfaces (e.g. glass) for around 2 weeks at 15ºC, but for only 4 days at 25ºC. Recent research indicates that leaf material is probably no longer infectious after dry storage for 3-4 weeks.

- Handle just the propagation cube and not the plant itself.

- If the previous crop was affected, it is preferable that new slabs are used, rather than steaming and re-using the old ones.

- Delay handling plants for as long as possible prior to stringing. Do not handle plants in the crop unless absolutely essential!

- Restrict workers to their own designated crop areas. Always work in the same direction.

- Wear disposable gloves at all times. Dispose of them when leaving an area and replace with new ones on return. Wash clothing regularly.

- Movement of staff, tools and equipment between different glasshouse blocks and nurseries should be avoided. If unavoidable, tools and equipment should be misted or wiped with a suitable disinfectant. Within a large house, have designated trolleys and tools for different areas.

- Restrict entry of visitors into the glasshouse.

- Regularly check for and remove any volunteer tomato seedlings within or around the glasshouse. Use disposable gloves and dispose of them after handling seedlings. Volunteer seedlings we tested within a house containing an affected crop tested positive for PepMV.

- Maintain a disinfectant foot and wheel dip (e.g. a large piece of matting soaked in disinfectant) at the glasshouse doorway. Current knowledge suggests that suitable disinfectants include trisodium orthophosphate (TSOP), Virkon S and Menno

Florades. Work evaluating a wide range of disinfectants for their effectiveness against PepMV in different situations is in progress.

- Ensure all staff thoroughly wash their hands at all meal and other breaks, before re-entering the crop.

Further information

MAFF website on new diseases: www.maff.gov.uk/planth/what.htm

Tim O'Neill of ADAS, Daphne Wright of CSL and Nicola Spence of HRI Wellesbourne are working on the disease in a new HDC project (PC 181), with the first report due in April 2001. Currently available to levy payers from the HDC (Tel: 01732 848383) are a Fact Sheet (12/00) entitled: *Pepino mosaic*-a new disease of tomato; and a translation of a Dutch publication on a hygiene protocol to reduce the risk of PepMV.