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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.

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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

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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.*

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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.

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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.

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