Project title:	Protected tomato: sources, survival, spread and disinfection of potato spindle tuber viroid (PSTVd)
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The results and conclusions in this report are based on a series of experiments conducted over one year. 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

I declare that this work was done under my 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

- Seedling bioassay to assess PSTVd viability developed
- In a commercial glasshouse in early autumn, PSTVd incidence increased by 74% over a 4-week period.
- PSTVd was detected on seed from infected fruit, but when grown on no plants were infected after 9 weeks.
- PSTVd detected in crop debris after an outbreak; the viroid survived in debris for at least 6 months.
- PSTVd in dried leaf sap remained infectious for at least 8 weeks (much longer than PepMV), but not 16 weeks.
- Efficacy of 7 disinfectants against PSTVd varies greatly; Horticide was best on glass and concrete.
- Sodium hypochlorite was effective against PSTVd in dried leaf sap on glass and concrete at 5,000 ppm, not at 1,250 ppm.
- The risk of PSTVd occurring in weeds and potatoes, and its spread by insects, is assessed.

Background and expected deliverables

In 2003, there was a single outbreak of potato spindle tuber viroid (PSTVd) in protected tomatoes in southern England; the first in the UK. The infection was successfully contained and eradicated by implementation of plant health quarantine measures. This project has used the opportunity presented to gather more information from the outbreak site to improve knowledge on the risk of this pathogen and help prevent further outbreaks in the future. It also allowed further investigation into the measures necessary to control this pathogen, permitting further refinement to optimise their performance, while reducing the impact on the grower.

Specific deliverables:
1. Recommendations on effective disinfectants
2. Specific information on the survival of PSTVd and how this will influence control measures
3. Information on the pattern of spread of PSTVd within an outbreak glasshouse and how this will influence plant removals at future outbreaks
4. The development of an efficient and reliable bioassay, which will be invaluable for monitoring PSTVd infections at any future outbreak or as part of any future scientific studies
5. Further information on the risks posed by PSTVd and associated advice that can be given from this.

Summary of the project and main conclusions

1. Spread of PSTVd within a tomato crop
   Monitoring the spread of PSTVd in a commercial glasshouse from isolated symptomatic tomato plants over a 4-week period revealed no increase in the number of plants showing symptoms. However, the proportion of infected asymptomatic plants increased from 2.7 to 4.7%. Spread was greater along rows (in both directions) than between rows, occurring up to 15 m distant from the symptomatic plant. Distribution of infected plants was discontinuous, with infection of adjacent plants rare. Lack of symptom expression may have been due to low temperature.

2. Seed transmission
   Seed taken from PSTVd infected tomato fruit tested positive for the viroid. When grown on for 9 weeks, none of the resultant 337 plants were found to be infected by PSTVd. Previous studies have reported rates of transmission between 2 and 23%.

3. Bioassay for PSTVd
   A bioassay to assess viability of PSTVd, using 'Rutgers' tomato seedlings, was developed. The test takes 3 weeks.

4. Sources of PSTVd in a glasshouse
   Following an outbreak in a commercial glasshouse and thorough disinfection with 3% sodium hypochlorite, PSTVd was only detected in plant debris, but not on swab samples taken from over 50 surfaces. This result contrasts with PepMV, which was found on many surfaces after an outbreak.

5. Survival in dried leaf sap
   PSTVd survived at least 4 weeks at 30°C on aluminium (in the light and dark) and on glass (in the light) and was able to infect plants. Survival on concrete was better in the dark than in the light. Survival on plastic appeared to be low. At both 15 and 25°C, PSTVd remained infectious for at least 8 and not 16 weeks, much longer than PepMV.

6. Disinfection
   Overall, Horticide was the most effective of seven disinfectants tested against PSTVd. Horticide, Jet 5, TSOP and Virkon S were all reasonably effective on glass. Horticide and Menno Florades were most effective on concrete. It was notable that Virkon S worked well on glass (after 5 mins contact) and not at all on concrete (even after 24h).

7. Effective sodium hypochlorite concentration for disinfection
   The current recommended treatment for PSTVd is sodium hypochlorite at 2-3% active ingredient (20,000–30,000 ppm). This very high concentration presents problems of user safety and corrosion. Against PSTVd in dried tomato leaf sap on glass and concrete, tests showed that the concentration could be reduced to 5,000 ppm hypochlorite (0.5%) and remain fully effective after 5 mins. At 1,250 ppm, (0.125%) the disinfectant gave poor control even after 1 hour.

8. Survival in leaf debris
   PSTVd survived at least 6 months in both dried and moist leaf debris. This is much longer than PepMV. Tests are continuing.

9. Weed hosts
   With the exception of avocado, all the natural recorded hosts of PSTVd are in the Solanaceae. Particular attention should be given to controlling solanaceous weeds (e.g. black and woody nightshade), as well as volunteer tomato seedlings, at any outbreak site.

10. Insect vectors
    Aphid transmission of PSTVd in potato crops has been recorded where there is co-infection with potato leafroll virus. The risk of insect transmission in UK tomato crops is considered to be very low.

11. Infection of potato
    The PSTVd isolate found in UK tomato was shown to be capable of infecting 4 out of 5 potato varieties tested. The actual risk of transmission between protected tomato and field-grown potato is considered to be very low.

12. PSTVd in New Zealand
    A summary of experience with PSTVd in New Zealand is given, and directions to website information (including symptoms and a code of practice).

Financial benefits

PSTVd is an important pathogen of tomato and potato crops and could cause losses of 50-60%; if it were to become established in the UK it would seriously damage production of tomatoes and potatoes.

An outbreak in a tomato crop can result in substantial financial cost. Control is effected primarily by removal of plants and a surrounding cordon-sanitaire. Statutory conditions are imposed by PHSI at sites where PSTVd is confirmed in England. Losses result from:
(1) cost of removal and disposal.
(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 overclothes) in efforts to prevent spread to other houses.
(5) potential inability to maintain supply to the customer (supermarket contracts).

Action points for growers

1. Be aware of the symptoms of PSTVd in tomato and pepper. See (www.vegfed.co.nz).
2. As a precaution, control Solanaceous weeds and volunteer tomato seedlings on and close to a nursery.

Science Section

1. Spread of PSTVd within a crop

Introduction

Although PSTVd is known to be mechanically transmitted in plant sap (e.g. by leaf to leaf contact of adjacent plants), little is known about the pattern or rate of spread in an infected tomato crop. Initial observations suggest that it does not spread rapidly down a row, as has been observed with *Pepino mosaic virus* (PepMV). The objective of this work was to map the spread of PSTVd over a 4-week period around single, visibly-affected plants.

Material & methods

Spread of PSTVd was assessed in a commercial crop of cvs Nectar and Rosa (plants grown under the brand name 'Rosa', but in fact the cultivar is 'Passion') in a glasshouse in Kent where the disease was first observed in late June 2003. Plants were examined for symptoms of PSTVd and leaf samples close to the plant head (second or third leaf from the growing point, around 8-10 cm in length) were collected and tested for PSTVd. Samples were collected on 18 September and 16 October 2003. In order to minimise the risk of cross-infection when sampling, labelled plastic bags were turned inside out over the hand and placed over the leaf to be sampled. The leaf was broken off through the bag, the bag turned right side out, pulled over the leaf and sealed. Samples were tested for PSTVd at CSL by TaqMan assay. Protective clothing and gloves were worn at all times in the glasshouse and changed at the end of each row.

On 18 September 2003, sampling was conducted in four areas:
1. Nectar, rows 7-9 (1 plant with symptoms in row 7, 8.3 m from main path)
2. Rosa, Rows 36-38 (1 plant with symptoms in row 37, 5.9 m from main path)
3. Rosa, rows 46-48 (1 plant with symptoms in row 48, 26.5 m from main path)
4. Rosa, rows 92-94 (1 plant with symptoms in row 93, 2.5 m from main path)

The crop was grown on rockwool slabs and plants were trained on the V-system i.e. each cube on the slab contained two plants, with one trained upwards in either direction so as to form a double row of plants above the single row of rockwool slabs. Sampling in each of the four areas was done in three rows of plants: the row containing the plant with visible PSTVd symptoms and one row either side. Each row was around 50 m long and contained around 150 plants. Intensive sampling (1 plant in every 3-5) was done for around 6 m either side of visibly affected plants, and for two areas extensive sampling (around 1 plant in 10) was done along the whole length of a row. The total numbers of plants sampled in each of the four areas were as follows:

Area (rows)	No. plants sampled intensively	Sampled row length (m)	No. plants sampled extensively	Row length (m)
1. Nectar (7-9)	20	13.5	20	50
2. Rosa (36-38)	21	13	0	50
3. Rosa (46-48)	20	14.6	15	50
4. Rosa (92-94)	15	8.5	0	50

Crop operations

The original infector plant in each of the four areas was left in during the monitoring period. No de-leafing or fruit picking was done in the trial areas. However, the crop was gone through twice by nursery staff to remove red fruit and thereby prevent seed falling to the ground, and once to stop and lower the plants.

Each plant was labelled at the first sampling by writing with permanent marker on the stem support hook. Most of the labelled plants were found at the second sampling but some labels had disappeared. Where this occurred, a sample was taken from the plant mid-way between two labelled plants. Distances between sampled plants were estimated by pacing.

Testing for PSTVd

Samples were tested by TaqMan PCR.

Results & discussion

At the second sampling, on 16 October 2003, monitoring area 4 (Rosa, rows 92-94) had died from lack of water and could not be sampled. No increase in the number of plants with symptoms of PSTVd was observed between 18 September and 16 October 2003 in the remaining three monitored areas. However, on both sampling dates PSTVd was detected in leaves taken from close to the head of some apparently healthy plants (Table 1).

The proportion of apparently healthy plants that tested positive for PSTVd increased from 2.7% on 18 September to 4.7% on 16 October. The mean distance between the visibly affected plant and symptomless infected plants in the monitored areas ranged 1.3 to 5.3 m on 18 September and from 2.3 to 5.1 m on 18 September. The greatest distance an infected plant was found from a visibly affected plant was around 15 m (Table 2). There was greater spread of the viroid within the double row containing a visibly affected plant than across pathways into plants of adjacent rows (Table 3).

Spread of the viroid is shown diagramatically in the Appendix. The distribution of infected plants was discontinuous, with no runs of three or more contiguous plants infected, and only rarely were two adjacent plants infected. Asymptomatic infected plants were not confined to just one side of the visibly infected plants, indicating spread had occurred both up and down the rows.

This study found a 74% increase over a 1-month period, from 2.7% to 4.7%, in the number of plants infected by PSTVd. None of the asymptomatic plants found to be infected with PSTVd on 18 September had developed obvious symptoms indicative of PSTVd by 16 October, suggesting a latent period of at least 4 weeks in certain conditions. The latent period in tomato is reported to be 2-3 weeks, with symptoms occurring once the temperature rises above 25°C, or a 24 h mean of 20°C (this temperature was reached in late March in 2004). Due to the presence of this disease and also PepMV and tomato mosaic virus (ToMV) elsewhere in the house, the grower reduced heating of the crop during the experiment. Symptom severity is reported to be considerably less once the temperature falls below 18°C, even if for just a few hours. This probably explains the lack of symptom expression by infected plants.

The increase in the number of plants testing positive for PSTVd between 18 September and 16 October may reflect spread that had already occurred before controls were put in place, the level of viroid being at undetectable levels on the first date.

The pattern of disease spread, with isolated plants affected up to 15 m distant from the symptomatic plant, suggest that the viroid is not as infectious as PepMV under the cool conditions in which the crop was grown between mid-September and mid-October. However, staff movements along the rows during this time were considerably less than in normal cultural operations, and it is possible that a greater degree of spread may have occurred had routine de-leafing and fruit picking been maintained. The exact means by which the viroid spread from one plant to another is unknown; it seems more likely to be by human activity than by leaf-leaf contact of adjacent plants, given the discontinuous pattern of infection along rows.

Table 1.1. Occurrence of PSTVd in asymptomatic tomato plants, cvs Nectar and Rosa – autumn 2003

Area*, variety And rows	No. samples positive/No. tested 18 September	16 October
1. Nectar (7-9)	4/157	7/157
2. Rosa (36-38)	2/70	4/69
3. Rosa (46-48)	0/134	6/134
4. Rosa (92-94)	5/45	-
Total	11/406 (2.7%)	17/360 (4.7%)

* 1 visibly affected plant in each area

Table 1.2. Spread of PSTVd in tomato over a one-month period from a single visibly affected plant in each of four areas

Area, variety and rows	Approximate distance between infected plants			
	Mean		Maximum	
	18 Sep	16 Oct	18 Sep	16 Oct
1. Nectar (7-9)	5.3	5.0	11.5	14.7
2. Rosa (36-38)	2.2	5.1	2.2	6.9
3. Rosa (46-48)	-	2.3	-	7.0
4. Rosa (92-94)	1.3	-	2.6	-

Table 1.3. Spread of PSTVd within and across rows of tomato, each area containing one symptomatic plant, over a one-month period – autumn 2003.

Area, variety and rows	No. samples positive/ No. samples tested			
	18 September		16 October	
	Within row	Across path	Within row	Across path
1. Nectar (7-9)	3/84	1/73	6/84	1/73
2. Rosa (36-38)	2/40	0/30	4/39	0/30
3. Rosa (46-48)	0/71	0/63	6/71	0/63
4. Rosa (92-94)	4/30	1/15	-	-
Total	9/225 (4%)	2/181 (1.1%)	16/194 (8.2%)	1/166 (0.6%)

2. Seed Transmission

Introduction

PSTVd is known to be transmitted via tomato seed. Many of the studies have shown that this transmission is true seed transmission, where the internal parts of the seed become infected, including the embryo. The rate of transmission is variable, with figures of between 2 and 23% reported. Infected tomato fruit was collected from the nursery with the outbreak of PSTVd, to investigate the rate of seed transmission from the UK outbreak.

Materials & methods

Seed from PSTVd infected fruit was extracted, sown in compost and grown at 30°C. Some of the seed was tested by TaqMan to confirm it was positive for PSTVd. After 9 weeks the tomato plants were tested by TaqMan for PSTVd.

Further studies were planned, using seeds extracted from fruit taken from artificially-infected plants. Unfortunately this proved impossible to achieve, as the inoculated plants failed to develop and never produced fruit.

Results & discussion

In total 337 plants were tested, but no PSTVd was detected in any of the plants. From these studies, it would indicate that the rate of seed transmission must be less than 0.3%. This is much lower than the transmission rates previously reported. It is worth noting that with potato, where more work on seed transmission has been conducted, quoted transmission rates do vary greatly between negligible and 100%. It is possible that the same variation in rates also occurs with tomato but insufficient studies have been carried out to date to demonstrate this.

It is not known why the rate of transmission is so low, but it may be due to the strain of the PSTVd found at the UK outbreak or due to the cultivar of the tomato involved (Passion). Interestingly limited studies conducted in New Zealand also failed to identify seed transmission in tomato infected with PSTVd. Given the high degree of similarity between the UK and NZ isolates, it is possible to speculate that there is a link to the strain found in both countries and seed transmissibility. However, there is no direct evidence to prove this and further studies would be required to investigate which factors actually affect seed transmission rates.

3. Risks of infection in the supply chain

Introduction

In the case of certain viruses (e.g. *Pepino mosaic virus*) there is evidence to suggest that the movement of infected fruit within the trade presents a significant risk to growing crops, especially when this material is handled in packing houses at glasshouse sites. Given that PSTVd is also a mechanically-transmitted agent, there is potentially a similar risk posed, especially if fruit from outbreak sites is allowed to be marketed.

Materials & methods

Fruit was taken from infected plants at the outbreak site and the flesh was tested using TaqMan. It was further intended to determine if infectious viroid could be picked up by handling infected fruit, by taking swabs from the outside of infected fruit, where the skin was not broken and inoculating tomato plants. However, it was impossible to complete this study, as by the time a suitable bioassay had been developed, the crop had been removed and infected fruit was no longer available.

Results & discussion

Testing showed that fruit taken from infected plants was indeed infected with PSTVd. While it was impossible to determine the risk associated with intact fruit, the overall risk of handling infected fruit is such that there should be a recommendation to destroy fruit from infected plants. However, this does not preclude the sale of fruit taken from uninfected plants in the same glasshouse/on the same site, provided that it is not sent to other sites growing tomatoes or other susceptible hosts and is sold direct to retail.

4. Effectiveness of procedures and products used for PSTVd control

4.1. Development of a bioassay

A bioassay was developed to assess viability of PSTVd. A bioassay is vital for studies to monitor PSTVd survival and disinfection, as it allows detection of infectious viroid. The conditions for the bioassay had to be optimised, including the growing conditions, the tomato variety used and the age of the plants for inoculation and testing.

The final bioassay used 9 day old 'Rutgers' tomato seedlings, which were inoculated with material by gently rubbing the leaves with a cotton bud, soaked in water and celite (an abrasive powder). These inoculated plants were kept in a growth room at 30°C with 16 hours light. They were tested by TaqMan after 3 weeks.

4.2. Sources of PSTVd in a glasshouse after an outbreak in tomato

Introduction

Studies on Pepino mosaic virus (PC 181) revealed widespread contamination of surfaces and equipment after an outbreak of the virus. In many instances the virus was present at transmissible levels. Similar information on the potential risk of PSTVd persisting in a glasshouse after an outbreak of the disease on tomato would help to inform decisions on cleaning and disinfection procedures after a crop is removed. The objective of the work described here was to determine the occurrence of PSTVd on plant debris, different surfaces and equipment within a glasshouse by collecting swab samples in an affected crop and testing them for PSTVd.

Materials & methods

A tomato glasshouse in Kent, where PSTVd was first observed in June 2003, was sampled on 16 October just before the crop was removed, and again on 25 November, after clean-up and disinfection following PHSI guidelines: the structure and equipment were disinfected with 3% sodium hypochlorite (30,000 ppm) thoroughly. Surfaces were swabbed using cotton buds dipped in ethanol (preliminary results showed this is the optimum storage buffer for preserving PSTVd on swabs). The swabs were placed in sterile Universal tubes containing 3-5 ml 50% ethanol and sent to CSL for testing. Any RNA was then extracted from the swabs by heating at 95°C in water and Chelex-100. This was then tested for PSTVd by TaqMan. In an initial experiment, dried tomato sap infected with PSTVd was swabbed with a cotton bud dipped in ethanol to confirm the effectiveness of the testing method.

In addition to swabs, samples were collected from inside the glasshouse, from tomato seedlings outside and from an adjacent tomato pack house. These were also sent to CSL for TaqMan testing.

Samples taken on 16 October 2003

There were three replicate swabs per Universal tube and four replicate locations per surface (i.e. 40 samples in total):

1. Heating pipes
2. Picking crates
3. Concrete pathways
4. Door chain/knob
5. Aluminium stanchions
6. Glass slide
7. Drip pegs
8. Picking trolley
9. Disinfectants
10. Rockwool slab/solution

Samples taken on 25 November 2003

<u>From area of glasshouse where crop was affected by PSTVd</u>

1. Door handles
2. Door mat (disinfectant)
3. Dripper peg
4. Roof support
5. Heating pipe
6. Polystyrene
7. Sitting trolley
8. Phone
9. Overhead pipe
10. Cleaning away trolley
11. Black drainage pipe
12. Heating pipe stand
13. Concrete pathway
14. Glass (near door)

<u>From packhouse</u>

15. Blue waste bin – opening end
16. Blue waste bin – tipping end
17. Waste bin
18. Grading bin
19. Large packing line
20. Packing line belt-debris
21. Packing line belt-debris
22. Packing crate (Israeli)
23. Waste bin
24. Floor
25. Packing crate
26. Drinking water point
27. Packing crate
28. Packing crate (Portuguese)
29. Floor
30. Waste bin
31. Blue bin – opening end
32. Blue bin – tipping end
33. Waste bin
34. Hand trolley
35. Hand washing equipment
36. Incoming produce room floor
37. Incoming produce room floor
38. Incoming produce room floor
39. Pipe base
40. Packing boxes (UK)
41. Packing boxes (UK)
42. Bobbins
43. Yellow skip
44. Compressed cardboard boxes
45. Picking crate (with soil in)
46. Transit trolley
47. Transit trolley
48. Grey wheely bin
49. Grey wheely bin
50. Door mat
51. Forklift wheel guards
52. Packhouse doors
53. Ship juice
54. Forklift tipper

From outside
Tomato seedling

From inside glasshouse
Debris sample, row 37 (heating pipe stand)
Debris sample, row 90
Debris sample, row 92 (behind large pipe at row end)
Debris sample, row 109 (behind large pipe at row end)

Results & discussion

No PSTVd was found in the samples taken on the 16 October. In the samples taken on 27 November, the test on a debris sample collected from row 92 gave a weak positive reaction. This was debris within the heating pipe support. All other samples were negative. A positive test by TaqMan indicates that part of the viroid genome has been detected; it does not necessarily indicate that it is infectious. As no bioassay was available at the time, it is not possible to demonstrate if this material was infectious. It was concluded that the result probably represented a very low concentration of part-degraded viroid.

Overall these results indicate that extensive contamination of the outbreak site had not occurred. This is in sharp contrast to similar studies carried out at PepMV outbreak sites, where infectious virus was found throughout the glasshouse on a wide range of surfaces and on much of the collected samples of debris etc. These contrasting results probably reflect the differences in distribution found between PSTVd and PepMV in standing tomato crops. Where PepMV is normally found at a high incidence (approaching 100% of plants infected), with the 2003 PSTVd outbreak only a small number of plants were found and hence the possibility of extensive contamination was greatly reduced.

4.3. Survival of PSTVd in dried sap on glasshouse structures

Introduction

PSTVd is known to be mechanically transmitted, therefore it is possible that dried sap can act as a source of infection. Information on the length of time PSTVd survives, on different surfaces and under different conditions, will help to provide guidelines on minimising carryover of the disease after an outbreak.

Materials & methods

PSTVd-infected tomato leaf was collected 3 weeks after inoculation and confirmed positive by TaqMan. The infected leaves were ground up in water (1:5 dilution) and the sap was spread onto plastic trays, glass slides, concrete and metal (aluminium) surfaces. These were then placed in a controlled environment cabinet, kept at 70% relative humidity, 30°C and 16 hours day length (glasshouse conditions). Half of each of the surfaces were kept in the light and the other half in the dark. At 24 hours, 48 hours, 1 week, 2 weeks and 1 month, swabs were taken from the different surfaces,

with cotton buds soaked in phosphate buffer and inoculated onto 2 Rutgers tomato plants for each replicate. After 3 weeks the tomato plants were tested by TaqMan for PSTVd.

A second survival experiment was also set up to assess the effect of temperature on survival of PSTVd. As before tomato plants infected with PSTVd were ground up in water (1:5 dilution) and the sap was spread onto glass slides. These were then placed in 2 incubators, one set at 15^{o}C and the other at 25^{o}C, both in the dark. At 24 hours, 48 hours, 1 week, 2 weeks, 4 weeks, 6 weeks, 8 weeks and 16 weeks swabs were taken from the glass, with cotton buds soaked in phosphate buffer and inoculated onto 2 Rutgers tomato plants for each rep. After 3 weeks the tomato plants were tested by TaqMan for PSTVd.

Results & discussion

The results achieved on the different surfaces in the light and dark at 30°C are shown in Table 4.3.1.1 and Table 4.3.1.2. The results achieved at the two different temperatures are shown in Table 4.3.2.

Table 4.3.1.1. Survival of PSTVd in tomato leaf sap on different surfaces in the dark.

	No. of indicator plants (out of 3) positive for PSTVd			
Time	Plastic	Glass	Aluminium	Concrete
24 hours	1	3	3	3
48 hours	1	3	3	3
1 week	0	1	3	2
2 weeks	0	1	1	3
1 month	0	0	1	0

Table 4.3.1.2. Survival of PSTVd in tomato leaf sap on different surfaces in the light.

	No. of indicator plants (out of 3) positive for PSTVd			
Time	Plastic	Glass	Aluminium	Concrete
24 hours	0	2	2	0
48 hours	0	1	2	1
1 week	1	1	3	0
2 weeks	0	0	1	1
1 month	0	1	1	0

Table 4.3.2. Survival of PSTVd in tomato leaf sap on glass at 15ºC and 25ºC in the dark.

Time	No. of indicator plants (out of 3) positive for PSTVd	
	15ºC	25ºC
24 hours	3	3
48 hours	3	3
1 week	3	3
2 weeks	3	3
4 weeks	3	3
6 weeks	3	3
8 weeks	3	3
16 weeks	0	0

The results show that PSTVd can survive for at least 4 weeks at 30ºC on aluminium (in the light and dark) and on glass (in the light). There is some evidence that PSTVd remains more infectious in the dark than in the light, especially on concrete. On plastic the viroid did not survive well in either the light or dark, with only one replicate out of 6 being positive after 1 week. This may actually be because the viroid is irreversibly binding to the plastic and it is not being recovered, rather than being degraded. The strong binding of viroids to plastics has been identified before and probably relates to their highly complex molecular structure; the same structure that also makes them very stable and resistant to degradation.

PSTVd survived well at both 15ºC and 25ºC. It remained infectious at 8 weeks, much longer than most viruses, including *Pepino mosaic virus*. Laboratory studies have shown that PSTVd is much more thermally-stable than many viruses.

4.4. Comparison of disinfectants

4.4.1. Alternative disinfectants to sodium hypochlorite

Introduction

Sodium hypochlorite is the current recommended treatment for PSTVd. However other disinfectants are much more commonly used in commercial glasshouses, therefore a range of disinfectants were tested over a time course to see which, if any, were effective against PSTVd.

Materials & methods

PSTVd-infected tomato leaf was collected three-weeks after inoculation and confirmed positive by TaqMan. The infected leaves were ground up in water (1:5 dilution) and inoculated onto concrete and glass and allowed to dry.

Eight disinfectants were used (Virkon S, Menno Florades, Citric acid, TSOP, Hortisept, Horticide (Unifect G)*, Panacide and Jet 5), at their label recommended rates. Each disinfectant was sprayed onto the PSTVd infected surfaces and swabs were taken with cotton buds dampened with phosphate buffer after 1 minute, 5

minutes, 30 minutes, 1hour, 6 hours and 24 hours. The cotton buds were immediately rubbed gently onto the leaves of 2 Rutgers tomato plants (wrapped individually to avoid cross contamination) to test the viability of the viroid. After 3 weeks the indicator plants were tested by TaqMan for PSTVd.

*Note: Unifect G has the same formulation as Horticide and hence was not tested.

Results & discussion

The results indicate that that the efficacy of the different biocides used varied considerably (Table 4.4.1.1 & 2). Overall Virkon S, Jet 5, TSOP and Horticide (Unifect G) were all reasonably effective against PSTVd in tomato leaf sap on glass, with Horticide being the most effective. Menno Florades and Horticide were the most effective disinfectants against PSTVd on concrete. Some disinfectants were effective against PSTVd on one surface but not the other, for example Virkon, from 5 minutes onwards works well on glass but is not effective on concrete. This may be because the disinfectant is inactivated on the concrete.

Table 4.4.1.1. Survival of PSTVd in tomato leaf sap on glass after treatment with the different disinfectants (numbers out of 3 replicates)

	No. of indicator plants positive for PSTVd						
Disinfectant	1 minute	5 minutes	30 minutes	1 hour	6 hours	24 hours	Total (all time points)
Virkon S	2/3	0/3	0/3	1/3	0/3	0/3	3/18
Jet 5	0/3	1/3	1/3	0/3	1/3	1/3	4/18
Menno Florades	3/3	3/3	2/3	1/3	1/3	3/3	13/18
Hortisept	2/3	3/3	2/3	3/3	3/3	1/3	14/18
Citric acid	3/3	2/3	0/3	1/3	0/3	0/3	6/18
TSOP	3/3	0/3	0/3	0/3	0/3	1/3	4/18
Horticide (Unifect G)	0/3	0/3	0/3	0/3	0/3	1/3	1/18
Panacide	2/3	3/3	2/3	1/3	3/3	2/3	13/18

Table 4.4.1.2. Survival of PSTVd in tomato leaf sap on concrete after treatment with the different disinfectants (numbers out of 3 replicates)

	No. of indicator plants positive for PSTVd						
Disinfectant	1 minute	5 minutes	30 minutes	1 hour	6 hours	24 hours	Total (all time points)
Virkon S	2/3	3/3	3/3	2/3	0/3	2/3	12/18
Jet 5	1/3	2/3	2/3	2/3	1/3	0/3	8/18
Menno Florades	0/3	1/3	1/3	1/3	1/3	0/3	4/18
Hortisept	1/3	0/3	3/3	2/3	1/3	2/3	9/18
Citric acid	3/3	3/3	3/3	2/3	3/3	3/3	17/18
TSOP	3/3	3/3	3/3	1/3	1/3	0/3	11/18
Horticide (Unifect G)	0/3	0/3	0/3	0/3	0/3	1/3	1/18
Panacide	1/3	1/3	3/3	2/3	1/3	0/3	8/18

It must be remembered that with this experiment an artificially high level of PSTVd was inoculated onto the different surfaces; much higher than would be found in a tomato glasshouse. Therefore this is worse case scenario and if a disinfectant was effective under these extreme conditions, it should be effective under glasshouse conditions.

Overall, from these results the most effective alternative disinfectant recommended for use on both glass and concrete would be Horticide.

4.4.2. Optimisation of sodium hypochlorite concentration

Introduction

Sodium hypochlorite is the current recommended treatment for PSTVd, at a concentration of 2-3% active ingredient. However, while this concentration is effective, it does present problems in terms of both user safety and corrosion, in particular on aluminium. Therefore a range of sodium hypochlorite concentrations were tested to find the optimum concentration for use against PSTVd.

Materials & methods

PSTVd-infected tomato leaf was collected three-weeks after inoculation and confirmed positive by TaqMan. The infected leaves were ground up in water (1:5 dilution) and inoculated onto concrete and glass and allowed to dry. Sodium hypochlorite was used at 2.5%, 0.5% and 0.125%. Each concentration of disinfectant was sprayed onto the PSTVd infected surfaces and swabs were taken with cotton buds dampened with phosphate buffer after 5 minutes and 1hour. The cotton buds were immediately rubbed gently onto the leaves of 2 Rutgers tomato plants to test the viability of the viroid. After 3 weeks the indicator plants were tested by TaqMan for PSTVd.

Results & discussion

The results shown in Tables 4.4.2.1. & 2 demonstrate that both 2.5 and 0.5% hypochlorite are fully effective at removing PSTVd contamination from glass and concrete surfaces. In contrast, the lower concentration (0.125%) was not very effective, performing very poorly on glass.

Table 4.4.2.1. Survival of PSTVd in tomato leaf sap on glass after treatment with the different concentrations of sodium hypochlorite.

Concentration of sodium hypochlorite	No. of indicator plants (out of 3 replicates) positive for PSTVd	
	5 minutes	1 hour
2.5%	0	0
0.5%	0	0
0.125%	3	2 (out of 2)*

* One plant died hence results given for only two replicates.

Table 4.4.2.2. Survival of PSTVd in tomato leaf sap on concrete after treatment with the different concentrations of sodium hypochlorite.

Concentration of sodium hypochlorite	No. of indicator plants (out of 3 replicates) positive for PSTVd	
	5 minutes	1 hour
2.5%	0	0
0.5%	0	0
0.125%	0	2

Overall, the results indicate that a reduction in the current recommended sodium hypochlorite concentration used from 2-3% down to 0.5% would still give very effective control, while significantly reducing the adverse effects of using such high concentrations of hypochlorite (e.g. user safety and metal corrosion).

4.5. Survival in dried and moist leaf debris

Introduction

Given that PSTVd is a mechanically-transmitted pathogen, information is needed on the risk of carryover of PSTVd in infected tomato debris from one growing crop to another. Tests were set up to determine how long PSTVd remains infectious in both moist and dried leaf material.

Materials & methods

Leaves collected from PSTVd infected tomato plants were stored a) dry and b) moist at room temperature. After 24 hours, 48 hours, 1 week, 2 weeks, 1 month, 2 months, 3 months, 6 months and 12 months a sample of the leaves was ground up in phosphate buffer and inoculated onto 2 Rutgers tomato plants for each rep. After 3 weeks the tomato plants were tested by TaqMan for PSTVd.

Results & discussion

The results show that PSTVd can survive in both dried and moist leaf material for at least 6 months (Table 4.5.1). This is much longer than with most viruses including *Pepino mosaic virus*. This implies that any leaf material left in the glasshouse after clean-up is a possible risk of infection to a new crop. This emphasizes the need for an effective and rigorous clean-up program after any outbreak of PSTVd.

Table 4.5.1. Survival of PSTVd in dried and moist tomato leaf debris.

	No. of indicator plants (out of 2) positive for PSTVd	
Time	Moist leaves	Dry leaves
24 hours	2	2
48 hours	2	2
1 week	2	2
2 weeks	2	2
1 month	2	2
2 months	2	2
3 months	2	2
6 months	2	2
12 months	On-going	On-going

5. To evaluate the risks posed by weed hosts and the potential for insects to act as vectors of PSTVd

5.1. Weed hosts

PSTVd has a limited natural host range including six different crop species: potato, tomato, pepper, pepino, aubergine, and avocado; in addition a range of naturally-infected wild *Solanum* spp. have been identified in Australia and India. Of these natural hosts, with the exception of avocado, all are members of the family Solanaceae. Based on this evidence it is possible that indigenous solanaceous weeds, such as black and woody nightshade, could become infected and act as reservoirs of infection. As a result it is certainly worth controlling any such weeds at outbreak sites in future. Indeed given the potential risks of harbouring other viruses, the control of weeds (in particular solanaceous ones) at tomato sites is highly recommended and should certainly be considered as best practice. In addition, to weeds volunteer seedlings should also be controlled. In New Zealand, it was strongly suspected that volunteer tomato seedlings arising from the previous season were acting as reservoirs of infection for subsequent crops. Again this should form part of any best practice hygiene protocol.

In addition to solanaceous weeds, the scientific literature lists a large number of experimental PSTVd hosts; which will become infected with PSTVd under laboratory conditions. While this indicates a theoretical risk, the actual risk is likely to be very low, unless optimum infection conditions are achieved. In the UK, this is extremely unlikely for plants growing outside of glasshouses.

5.2. Insect vectors

In general, viroids are not transmitted by insect vectors. However, the one major exception to this is PSTVd, where efficient aphid transmission has been demonstrated in potato crops, co-infected with *Potato leafroll virus* (PLRV). In fact in China, it is considered to be a major factor for the spread of PSTVd in potato crops in certain regions. However, while there is an obvious potential risk, in the context of tomatoes within the UK, the actual risk is low. Within the UK, the incidence of PLRV in commercial potato crops is now actually very low and as UK tomato crops are glasshouse-grown and relatively aphid-free, the incidence of PLRV in tomato is very low (insignificant?). Hence for insect transmission of PSTVd to become a major issue within the UK industry, there would have to be a significant increase in both the aphid populations and PLRV incidence in the future. There is no reason to suspect or suggest that this will happen.

6. Risk assessment with respect to potatoes

Introduction

In the UK, potatoes are a major crop, worth around £500M per annum. As potato is a well-known natural host for PSTVd, it is necessary to ascertain the risk posed to field-grown potato crops by PSTVd-infected protected tomato crops.

Material & methods

Five of the most popular potato varieties grown in the UK, representing the three main crop types (i.e. first early, second early and main crop), were selected. Plants of each variety (10 pots of each) were mechanically inoculated with PSTVd, by grinding infected tomato sap in phosphate buffer with a small amount of celite added. The different varieties used were:

Maris Bard	(first early)
Maris Piper	(main crop)
Nadine	(second early)
Estima	(second early)
Pentland Dell	(main crop)

The potatoes were kept in a controlled environment cabinet, kept at 70% relative humidity, 30°C and 16 hours day length. The inoculated plants were tested by TaqMan for PSTVd after 4, 8 and 12 weeks.

Results & discussion

The results clearly show that the PSTVd isolate found in tomato in the UK is indeed capable of infecting a range of potato varieties (Table 6.1). This rules out the possibility that the isolate obtained from the UK outbreak is a specific tomato-adapted strain. In four out of the five varieties tested, infection was detected after only 4 to 8 weeks post-infection. In contrast, the variety Nadine remained uninfected after 12 weeks, despite being challenged with the same titre inoculum and being maintained under the same optimal conditions. As a result, this finding is potentially interesting and warrants further investigation.

Table 6.1. Potato varieties infected with PSTVd.

Variety	No. of infected plants detected by TaqMan (tested in 2 bulks of 5 plants each)		
	Week 4	Week 8	Week 12
Maris Bard	2/2	2/2	2/2
Maris Piper	0/2	2/2	2/2
Nadine	0/2	0/2	0/2
Estima	2/2	2/2	2/2
Pentland Dell	2/2	2/2	2/2

Overall these results indicate that there is indeed a risk posed to potato crops from PSTVd-infected tomato crops. Given this fact, careful consideration should be given to the possibility of cross-infection if any future PSTVd outbreaks should occur. As a result precautions should be put in place to prevent the accidental infection of potato crops within close proximity of any outbreak. This should involve ensuring that staff are fully aware of the potential for cross-infection. This advice should also be extended to other solanaceous crops, including pepper, aubergine, pepino and petunia.

However, while there is the definite potential for PSTVd to jump from tomato into potato, the actual risk of this occurring is probably extremely low. Given that transmission by vectors is highly unlikely and that in the UK there is invariably physical and spatial separation of commercially-grown tomato and potato crops, the probability of a direct link allowing mechanical transfer of viroid between the two types of crop is very low. This risk would be further reduced given the environmental conditions found in UK field crops, which are typically sub-optimal (e.g. too cool with reduced light levels) for viroid replication.

7. PSTVd in New Zealand

PSTVd was first observed on tomatoes in New Zealand in May (autumn) 2000. The disease was found in a glasshouse crop of cv. Daniella around 2-3 months after planting. Symptoms were confined to plant tops and consisted of leaf inter-veinal chlorosis, downward curling of leaf margins and brittleness. Around 10% of the crop was affected. A survey of tomato glasshouse crops throughout New Zealand revealed two further infected sites close to the original one in Auckland, and a further one in Nelson, South Island (around 300 miles south). It was considered that the disease was probably introduced into the glasshouses by use of infected seed.

Further outbreaks in tomato occurred in subsequent years. The disease was reported to show symptoms in most varieties where it was confirmed, including Aranca, Campari, Excel and Flavorine (earlier work in Canada has indicated that some tomato varieties infected with PSTVd do not show symptoms).

In November 2001, the viroid was detected in glasshouse peppers, close to Auckland, the first report of PSTVd infecting peppers in the world. Symptoms were wavy leaf margins and smaller fruits. Between April and June 2002, a survey was made of 59 tomato sites and 41 pepper sites throughout New Zealand to determine the extent of infection. Each site was inspected for symptoms and random samples were collected and tested for PSTVd. PSTVd was not detected at any of the 59 tomato sites (although it was still present at the original tomato outbreak site), but it was detected on three pepper nurseries. Varieties infected were Spirit, Special and Fiesta.

In studies of the disease at the Ministry of Agriculture & Forestry (MAF) Auckland, it was found that both the flesh and seeds of fruits from infected tomato and pepper plants tested positive for PSTVd, using a molecular method (RT-PCR). When seeds from infected plants were sown however, leaves from the seedlings tested negative for PSTVd. The failure of PSTVd to transmit to seedlings may have been because the seed coat was infected and not the embryo. It has been shown elsewhere that PSTVd is seed-transmitted in tomato, with infection levels as high as 25-30%. No method for disinfecting seed is currently available.

The New Zealand Vegetable and Potato Growers Federation (Wellington) (www.vegfed.co.nz) and the Vegetable Industry PSTVd Technical Advisory Group, have produced a New Zealand Code of Practice for the Management of PSTVd in Greenhouse Tomato and Capsicum Crops. The document contains information on:

- Background information on PSTVd
- Symptoms in tomato (www.vegfed.co.nz/about/12_research)
- Symptoms in pepper (www.vegfed.co.nz/about/11_research)
- Code of practice
    - responsibility
    - monitoring
    - containment of suspect and confirmed PSTVd
    - restrictions on sale
    - measures to prevent spread
    - procedures for disinfection between crops
    - record keeping
    - compliance monitoring
- Appendix on suggested disinfectants

The appendix notes that for several disinfectant products there is little scientific information available to support the efficacy of these products. It is recommended 1% sodium hypochlorite (10,000 ppm) as the most effective, practical and readily available disinfectant. It is also inexpensive and quick acting. However, care is needed in handling – it can harm hands and eyes (COSHH assessment required) and is corrosive. It is recommended that spraying sodium hypochlorite solutions be avoided as this produces aerosols that can be inhaled.

References

Elliott DR, Alexander BJR, Smales TE, Tang Z & Clover GRG (2001). First report of Potato spindle tuber viroid in New Zealand *Plant Disease* **85**, 1027.

Lebas BSM, Elliott DR, Orchoa-Corona FM, Tang J, Alexander BJR (2003). Delimiting survey for Potato spindle tuber viroid on tomato and capsicum in New Zealand Greenhouses. *Proceedings 8th International Plant Pathology Congress, Christchurch, New Zealand* (abstract) **2**, 267.

Anon (2003). New Zealand Code of Practice for the Management of Potato spindle tuber viroid (PSTVd) in greenhouse Tomato and Capsicum Crops. New Zealand Vegetable & Potato Growers Federation, Wellington (www.vegfed.co.nz).

8. Summary of previous studies on disinfection of PSTVd and related viroids

Table 8.1 summarises the results of tests using disinfectants available in the UK, or of products that have recently been available. The method commonly employed by the researchers was to contaminate a knife, by cutting through a known infected plant (Garnsey *et al*., 1997, Roistacher *et al*., 1969; Timmermann *et al*., 2001), or dipping a knife in a viroid suspension (Singh *et al*., 1989), then immediately dip the knife in disinfectant. After immersion for the relevant duration, the knife was then used to cut healthy indicator plants and the number of plants which subsequently developed viroid symptoms was assessed. Additionally, Singh *et al*., (1989), devised a severe test in which nucleic acid extracts (50 µl) containing several strains of PSTVd were mixed with disinfectant solutions (10 µl) and tested after incubation for defined periods.

For equipment contaminated by cutting through infected plants, only two materials were found to be effective, sodium hypochlorite (at concentrations of 2,000 ppm for 1 second for *Citrus exocortis viroid* (CEV) and at 10,000 ppm for 5 seconds for PSTVd) and Menno Florades (benzoic acid). Disinfectants found to be ineffective were formaldehyde, TSOP, ethanol, Lysol (a phenolic) and chlorine dioxide. Heat was ineffective, even at 150°C for 4-8 seconds, from a propane blowtorch.

When a severe challenge was given, by mixing the disinfectant with several strains of PSTVd, sodium hypochlorite at 10,000 ppm for 15 seconds was ineffective, though treatment at 30,000 ppm for the same time was effective.

Recent studies from the Netherlands, comparing disinfection using PSTVd-infected potato sap has indicated that concentrations of 1% hypochlorite are effective (Linda Kox, Pers. Comm.)

References

1. Garnsey SM & Whidden R (1971). Decontamination treatments to reduce the spread of citrus exocortis virus (CEV) by contaminated tools. *Proceedings Florida State Horticultural Society* **84**, 63-7.

2. Roistacher CN, Calavan EC & Blue RL (1989). Citrus exocortis virus – chemical inactivation on tools, tolerance to heat and separation of isolates. *Plant Disease Reporter* **53**, 333-6.

3. Singh RP, Boucher A, Somerville TH (1989). Evaluation of chemicals for disinfection of laboratory equipment exposed to potato spindle tuber viroid. *American Potato Journal* **66**, 239-45.

4. Timmermann C, Muhlbach HP, Bandte M & Buttner C (2001). Control of mechanical viroid transmission by the disinfection of tables and tools. *Mededelingen Factulteit Landbouwwettenschapen Univ. Gent* **66**, 151-6.

Table 8.1 Summary of tests to determine the effectiveness of chemical disinfectants and heat treatment for disinfection of PSTVd or *Citrus exocortis viroid* (CEV).

Disinfectant	Active ingredient	Conc. of product used	Conc. of NaOCl	Exposure time	Effective (✓) or ineffective (X)	Reference
		%	Ppm			
PSTVd knife dip						
Menno-Florades	Benzoic acid	1	-	1 min	X	4
		2	-	1 min	✓	4
		3	-	30 Sec	✓	4
Bleach (5.25% ai)	Sodium hypochlorite	1	10,000	5 Sec	✓	3
		2.5	25,000	5 Sec	✓	3
		5.25	52,000	5 Sec	✓	3
PSTVd severe test						
Bleach (5.25% ai)	Sodium hypochlorite*	1	10,000	15 Sec	X	3
		3	30,000	15 Sec	✓	3
Various	Chlorine dioxide	-	-	300 Sec	X	3
CEVd knife dip						
Bleach (5.25% ai)	Sodium hypochlorite	0.25	2,500	2-3 Sec	✓	1
		0.5	5,000	2-3 Sec	✓	1
Formalin (37% ai)	Formaldehyde	2	-	2-3 Sec	X	1
TSOP	TSOP	10	-	2-3 Sec	X	1
Ethyl alcohol	Ethyl alcohol	95	-	2-3 Sec	X	1
Bleach (5.25% ai)	Sodium hypochlorite	0.26	2,000	1 Sec	✓	2
		0.53	5,300	1 Sec	✓	2
Formalin	Formaldehyde	3	-	1 Sec	X	2
TSOP	TSOP	2	-	1 Sec	X	2
Ethyl alcohol + flame	Ethyl alcohol	95	-	1 Sec	X	2
Lysol	A phenolic	1	-	1 Sec	X	2
Propane blow torch (150°C for 4s)	Heat	-	-	4-8 Sec	X	2

*Found to be as effective at pH 7.0 as at pH 11.3.

Technology transfer

1. An update of PSTVD, PepMV and potential new virus threats to the UK. Presentation at the HDC/HRIA/TGA Tomato Conference 2004 (Rick Mumford and Nicola Spence).
2. Project update to TGA Technical Committee, 3 September 2004 (Rick Mumford).
3. Studies on potato spindle tuber viroid. Presentation to PHSI Conference, 12 January 2005 (Rick Mumford).

Appendix 1

