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# Virus diseases of Orchids

ORSV + CyMV coinfection

https://www.pbase.com/schnitz/virus_in_orchids

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26th July 2023 - Cymbidium Orchid Club of South Australia, Adelaide

# Why are viruses important?

Virus infections can cause unsightly symptoms (markings) on orchid leaves, & occasionally on flowers too.

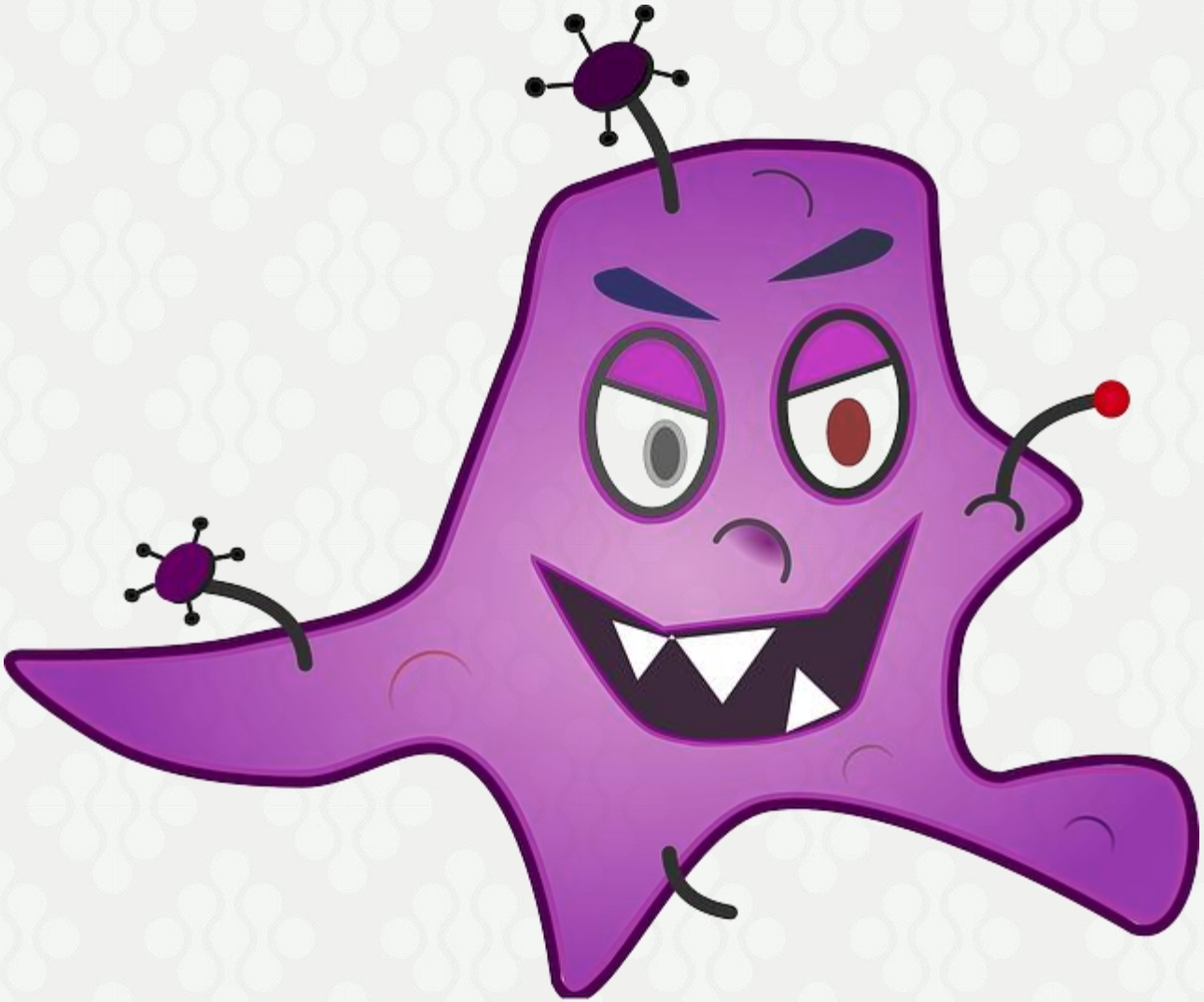
They also reduce plant vigour & can affect flowering.

# Why are viruses important?

Viruses are incurable.

- There are no treatments that can be applied that will eliminate virus infections.
- Unlike humans, plants do not produce antibodies.
- Rather prevention of infection and disease spread is the key to avoiding virus problems.

# What are plant viruses?



https://www.Clipartkey.com/search/germs/

# What are plant viruses?

In Roman times the term "virus" described:

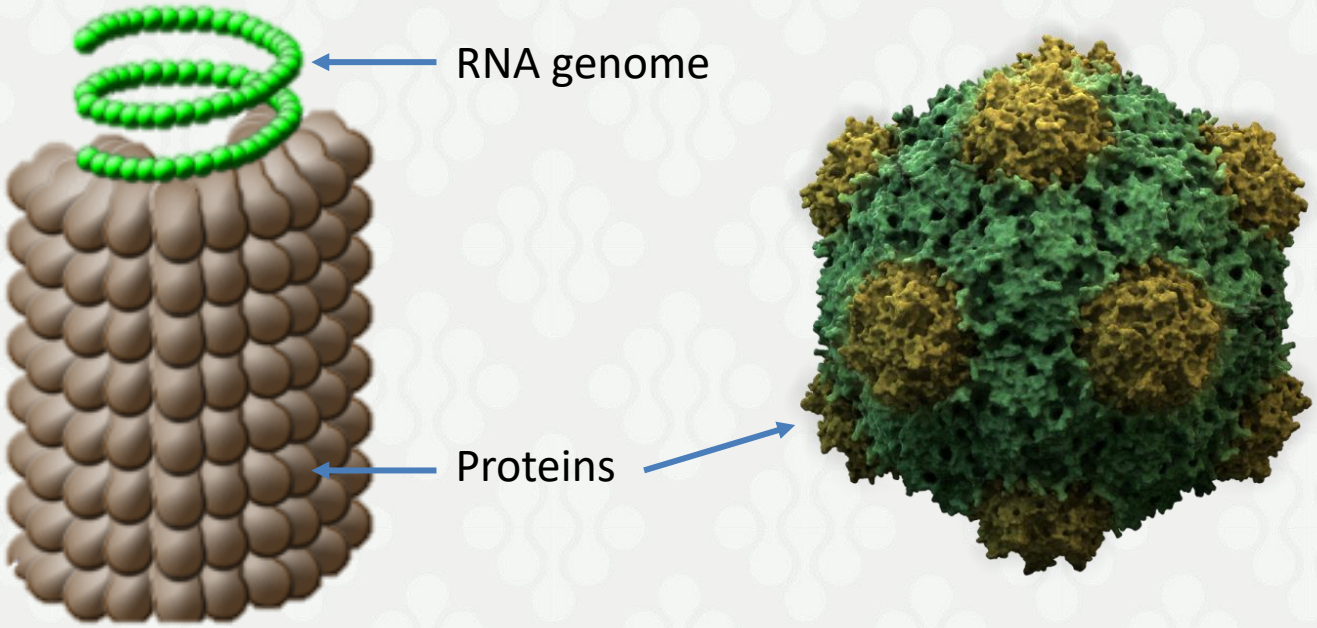
*"poison, venom, the rammish smell of armpits, water matter, whitish, yellowish & greenish at the same time which issues out of ulcers and stinks very much, being induced with eating and malignant qualities"*

# What are plant viruses?

1. They are very, very small
   (sub microscopic)
2. They are obligate plant parasites
   (they can only reproduce in an infected plant)
3. They are quite simple entities
   (and come in different shapes)
4. They are at the boundary of life itself

# What do they look like?

They are simply made up of a string of RNA nucleic acid (virus genes) covered in protein




https://www.old.amu.edu.az

https://www. en.wikipedia.org/wiki/Secoviridae

# What is a plant virus?

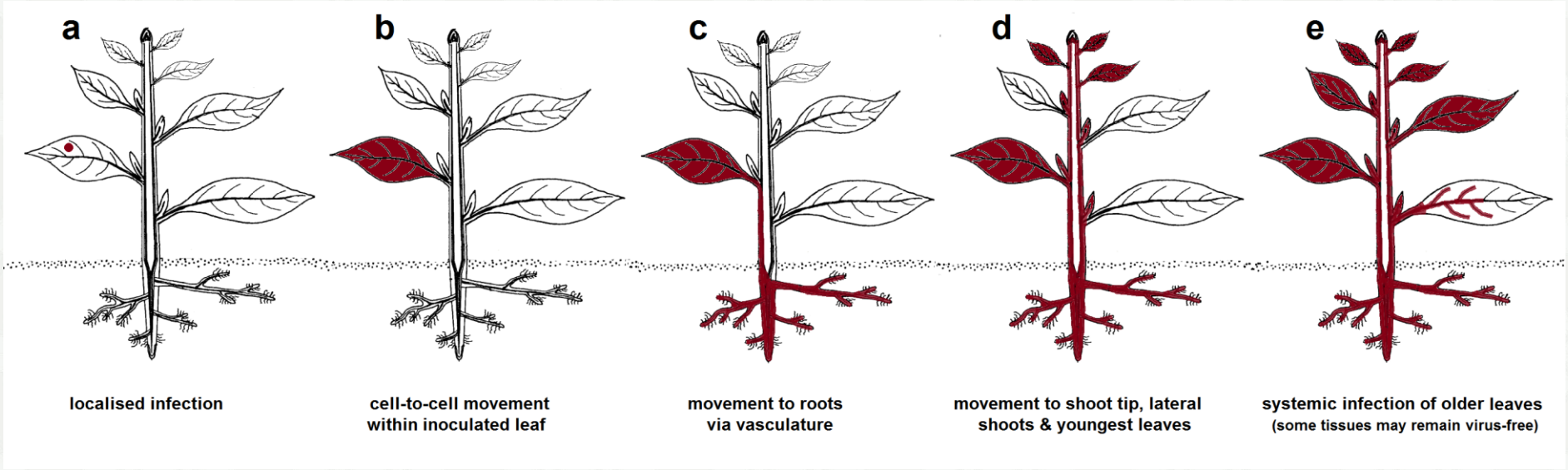
"a virus is a piece of bad news wrapped up in protein"

Jane & Peter Medawar

# How do they infect plants?

Viruses get inside & replicate in plant cells.

They then move systemically throughout the plant between cells & in the phloem.




Wilson CR (2014) Applied Plant Virology, CABI

# What viruses affect orchids?

There are over 30 different viruses recorded from orchids.

Confirmation of some of these is still required
(they may be different names for the same virus)

It is likely to be an underestimate
(as those that do not cause obvious symptoms have probably been largely ignored).

# What viruses affect orchids?

| Virus Genera | Number of species | Example |
|---|---|---|
| Carmovirus | 1 | Carnation mottle virus |
| Closterovirus | 1 | Dendrobium vein necrosis virus |
| Cucumovirus | 1 | **Cucumber mosaic virus** |
| Nepovirus | 1 | **Tomato ringspot virus** |
| Potexvirus | 1 | **Cymbidium mosaic virus** |
| Potyvirus | 13 | **Ceratobium mosaic virus** |
| Rhabdovirus | 2 | **Orchid fleck virus** |
| Tobamovirus | 1 | **Odontoglossum ringspot virus** |
| Tobravirus | 1 | **Tobacco rattle virus** |
| Tombusvirus | 1 | Cymbidium ringspot virus |
| Tospovirus | 3 | **Capsicum chlorosis virus** |

# Which are the most common?

Several international surveys suggests ORSV and to a lesser extent CyMV are common in orchids with OFV very rare.

In Australia however, OFV is generally more common than CyMV.

| | NSW (1989) | TASAG-ELISA (Australia wide: 1991-1999) |
|---|---|---|
| **CyMV** | 8.7% | 15.5% |
| **ORSV** | 61.9% | 63.4% |
| **OFV** | 29.4% | 21.1% |

Also, a survey of 100 plants from two Australian orchid collections showed 1/3$^{rd}$ were infected with *Ceratobium mosaic potyvirus* (with a few additional potyviruses detected).

# This year's data

172 orchid leaves have been sent to TASAG testing services so far this year.

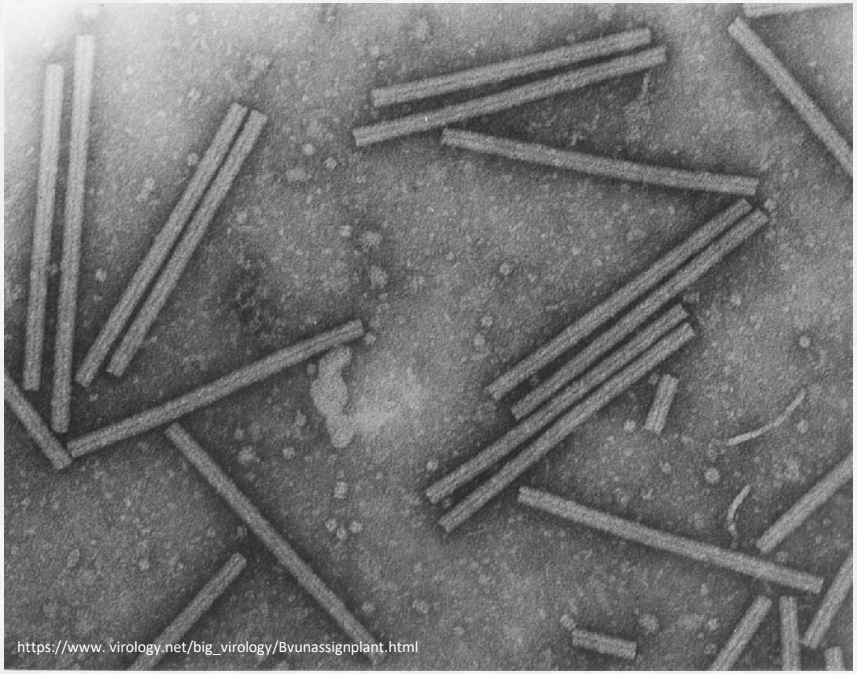
| | QLD | NSW | SA | VIC | TAS | Total |
|---|---|---|---|---|---|---|
| OFV | 1 | 6 | 10 | 5 | 0 | 22 (51%) |
| ORSV | 2 | 5 | 6 | 1 | 2 | 16 (37%) |
| CymMV | 0 | 2 | 0 | 1 | 2 | 5 (12%) |
| Total samples submitted | 21 | 85 | 27 | 30 | 9 | |

Most positive samples were symptomatic, but a few asymptotic samples were also infected.

Potyviruses were also occasionally detected.

# Common orchid viruses

## *Odontoglossum ringspot virus* (ORSV)




Syn: *Tobacco mosaic virus* – O

First reported in the USA in Odontoglossum in 1951

Particles are: 300 nm x 18 nm

(5% RNA; 95% protein)

# *Odontoglossum ringspot virus*

https://pbase.com/schnitz/image/65905371
https://m.cnhnb.com/xt/article-1931.html
https://archivo.infojardin.com/tema/la-orquidea-un-tesoro-de-la-naturaleza.172823/page-3
https://commons.wikimedia.org/wiki/File:Tobacco_mosaic_virus_symptoms_orchid.jpg

# *Odontoglossum ringspot virus*

Very common orchid virus world-wide

Natural host range: various orchid species
but can also infect wider range of plants (9 families)
(e.g. tobacco, Zinnia, Chenopodium).

Symptoms: can be highly variable, and some infected plants may be asymptomatic
(but will still have reduced vigour and can be a source of infection for other plants)

# *Odontoglossum ringspot virus*

This is the most infectious orchid virus

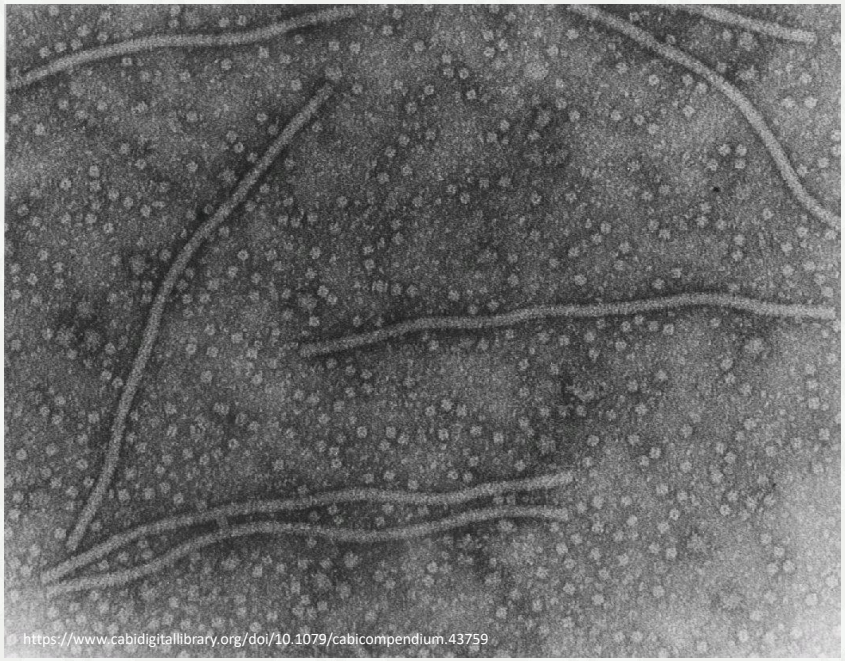
It is a very stable virus and can survive on surfaces for many years (in dried leaf for >100 years)

It is naturally spread by:

- vegetative propagation of infected plants
- plant-to-plant contact
- cutting, pruning, plant handling (contaminated tools)
- pollen can be infected
- It is not seed borne – but there could be possible seed contamination

# Common orchid viruses

## *Cymbidium mosaic virus* (CyMV)




First reported in Cymbidium in USA in 1950

Particles are: 480 nm x 13 nm

(6% RNA; 94% protein)

# *Cymbidium mosaic virus*

https://pbase.com/schnitz/image/65905374
https://archivo.infojardin.com/tema/la-orquidea-un-tesoro-de-la-naturaleza.172823/page-3
http://www.cyberwildorchid.com/products/example2/cultivation/disease/disease.html

# *Cymbidium mosaic virus*

Common orchid virus world-wide

Natural host range: various orchid species
but can also infect wider range of plants (9 families)
(e.g. rice, cucumber, nasturtium, Jimson weed).

Infected plants almost always show a leaf mosaic, but some plants can still be infected asymptomatically.


It can take time (weeks/months) for systemic symptoms of infection to be expressed.

Vigour is reduced in infected plants.

# *Cymbidium mosaic virus*

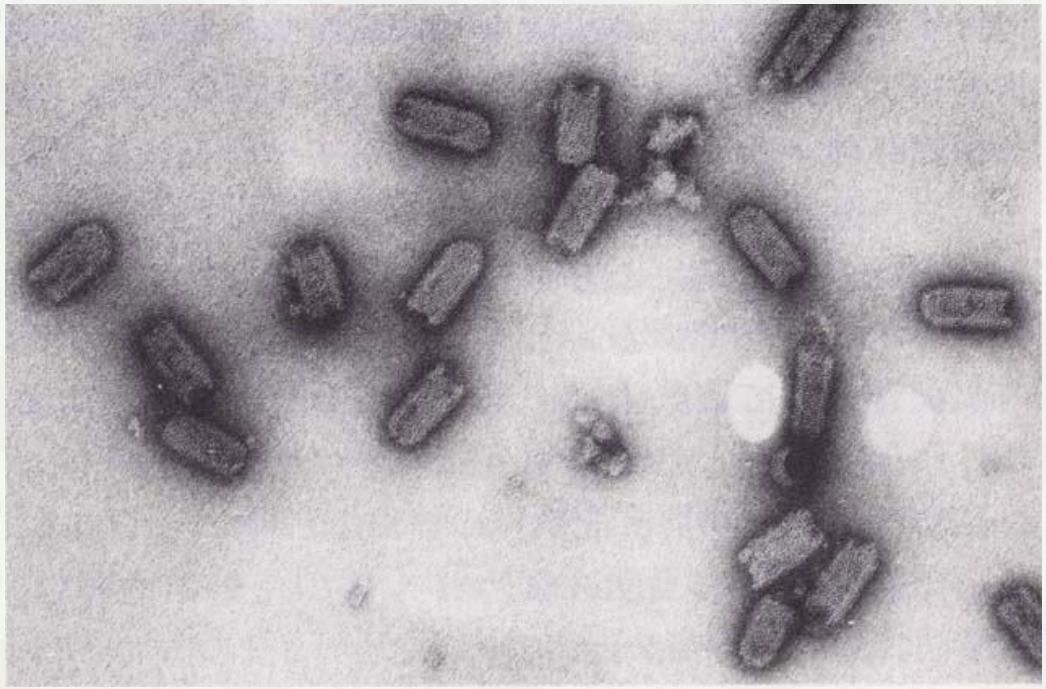
It is a moderately stable virus
(can survive for days outside of a host plant)

It is naturally spread by:

- vegetative propagation of infected plants
- plant-to-plant contact
- cutting, pruning, plant handling (contaminated tools)
- pollen can again be infected
- It is not seed-borne
- It was shown (experimentally) to be spread by a chewing insect (cockroach)

# Common orchid viruses

## *Orchid fleck virus* (OFV)



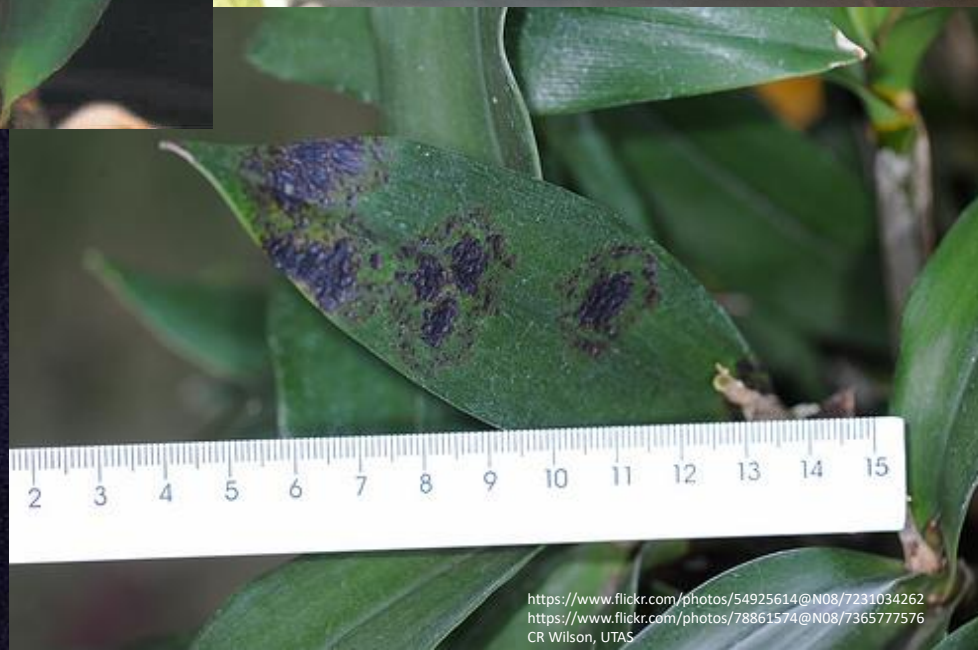
First reported in Japan in Cymbidium in 1969

Particles are:

105-150 nm x 40-50 nm

very unstable particles

# Orchid fleck virus



https://www.flickr.com/photos/54925614@N08/7231034262
https://www.flickr.com/photos/78861574@N08/7365777576
CR Wilson, UTAS

# Orchid fleck virus

This is common in Australia but less so elsewhere.

There are actually two orchid fleck viruses
(they differ in the parts of the plant cell they infect, but cause the same symptoms).

There are also related, but distinct, viruses that infect citrus & coffee.

Natural host range: in a wide range of orchid species
(it can infect other species, such as faba bean and Chenopodium, but in these other hosts infection is usually not systemic).

Infected cymbidium can show chlorotic and/or necrotic flecking and ring patterns.

# *Orchid fleck virus*

It is a very unstable virus

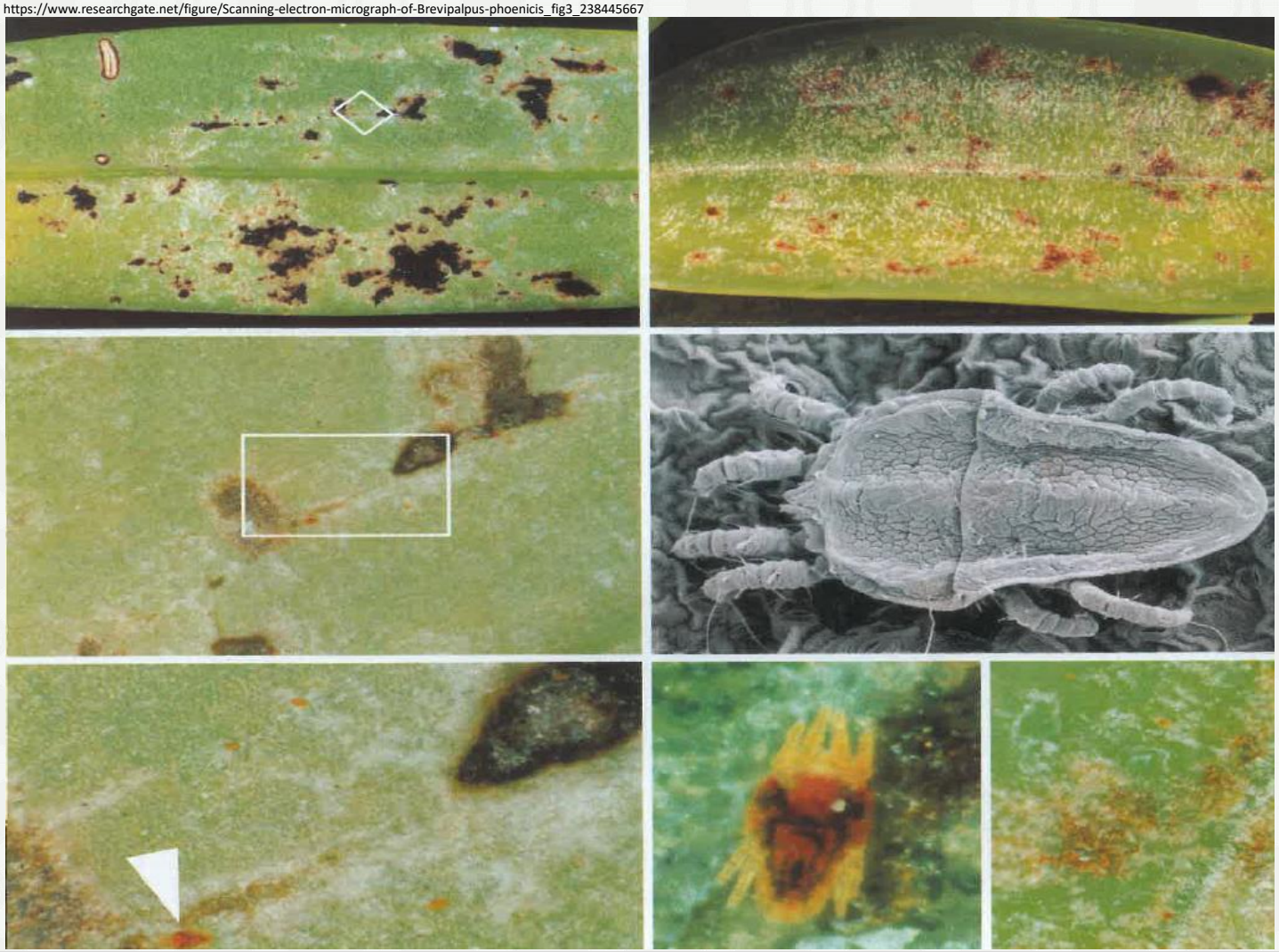
It is naturally spread by:

- false spider (flat) mite vectors (*Brevipalpus* spp.)
- vegetative propagation of infected plants
- plant-to-plant contact (but not easily)
- cutting, pruning, plant handling (but not easily)
- It is not seed-borne

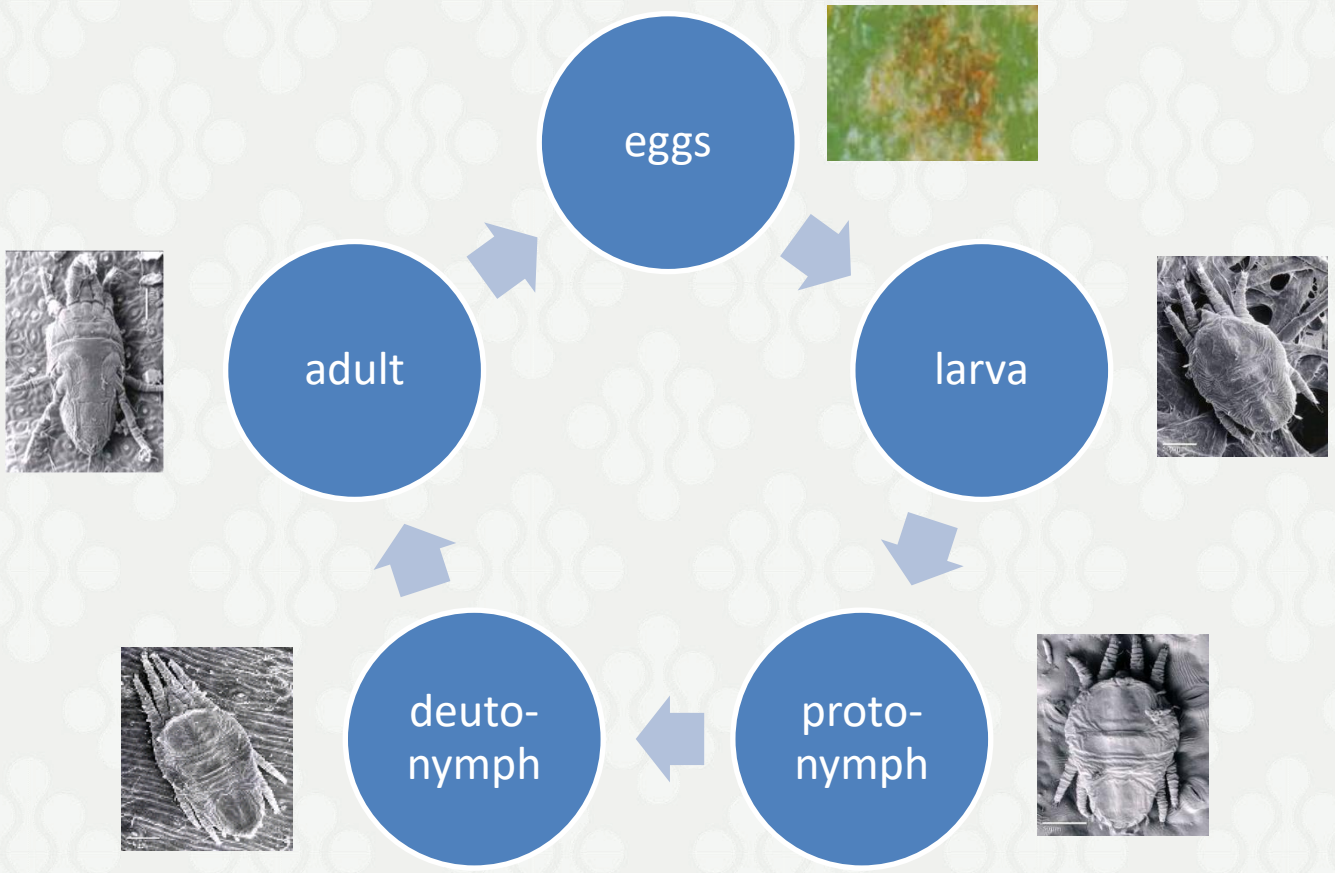
Spread by contact requires high temperatures (<30°C) and is easier to non-orchid plants.

# *Brevipalpus californicus*
## (false spider mite)

R Ali, UTAS
https://www.researchgate.net/figure/Scanning-electron-micrograph-of-Brevipalpus-phoenicis_fig3_238445667



# *Brevipalpus* lifecycle




Eggs (50-60 per female) take up to 3 weeks to hatch. Immature stages take 5-6 weeks to become adults. Between stages they exist as chrysalis glued to plants.

# Mites as virus vectors

OFV can be transmitted by at least two *Brevipalpus* spp. (*B. californicus* and *B. yothersi*), but not by them all.

Transmission occurs with nymphs and adults (but not larva)

Once the mites pick up the virus, they will probably carry it for their lifetime

They need at least 30 mins feeding to acquire and transmit the virus (the longer the time the more transmission events)

# Mites feeding

*Brevipalpus* mites naturally infest >1000 plants species.

They feed along midribs of leaves and then move outwards

They puncture the leaf surface and suck out the plant sap.

This results in leaves appearing mottled & silvery if in high enough numbers.

They do not produce webbing (like spider mites)

# Mite populations & movement

*Brevipalpus* produces large populations in hot & humid conditions & tend to hide in shaded areas on the plant

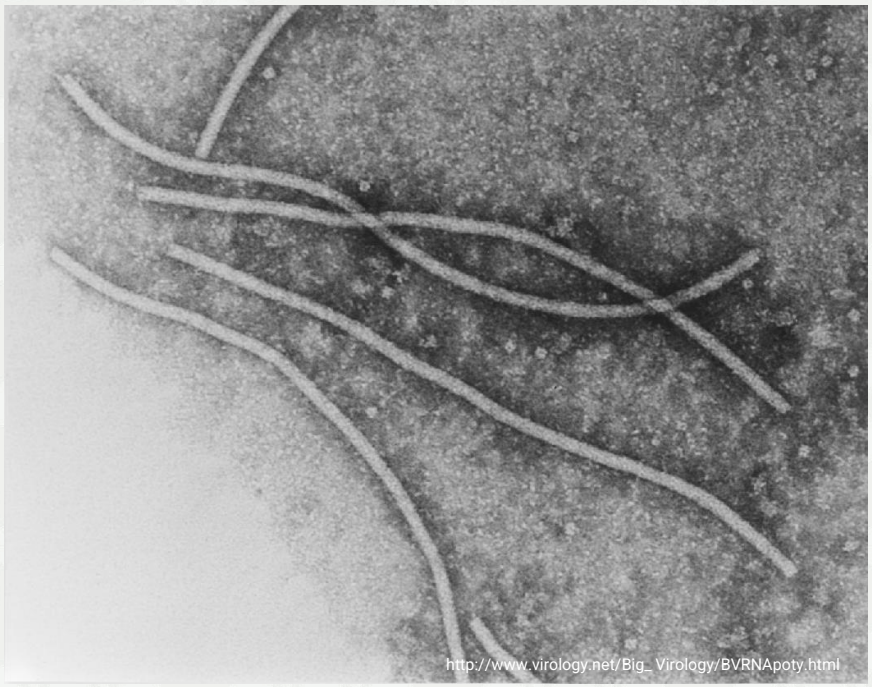
Mite movement by walking is not regarded as important, rather they “lift off” from leaf surfaces & move with the wind

Aerial movement of mites can be stimulated by overcrowding & host plant senescence or death

They can also be transported on infested plants & clothing

# Common orchid viruses

## Various orchid infecting potyviruses




*Ceratobium mosaic virus*
*Pterostylis virus Y*
*Clover yellow vein virus*
*Dendrobium mosaic virus*
*Bean yellow mosaic virus*
*Vanilla mosaic virus*
*Turnip mosaic virus*
*etc.*

# Potyviruses

A number are found in Australia

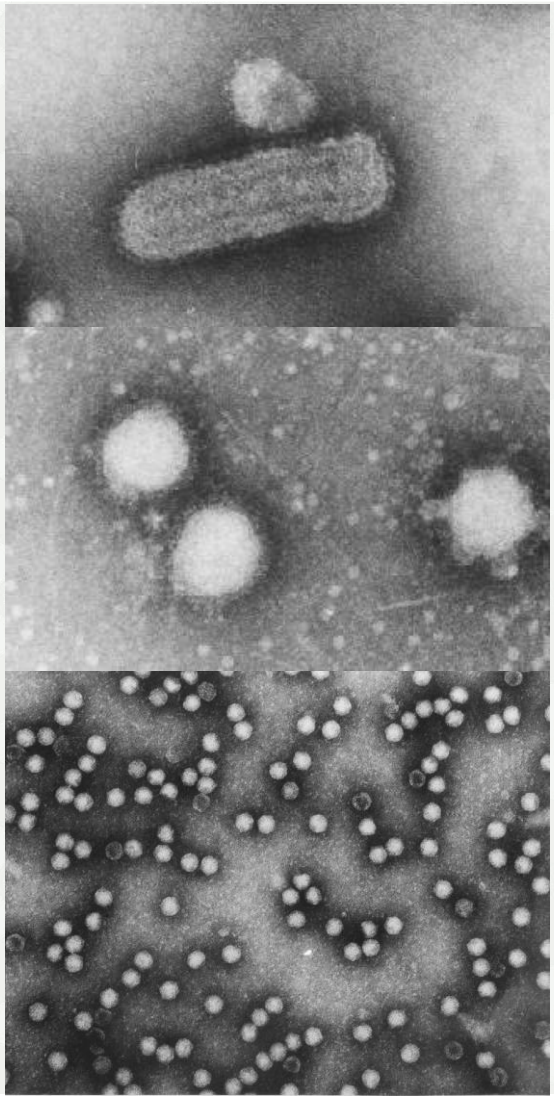
CerMV found in 1/3rd of orchids surveyed in two Australian collections

Some potyviruses have wide host range (BYMV, TuMV)

Naturally spread by:

- aphids
- cutting, pruning, handling of plants
  (but less efficiently than ORSV, CyMV)

# Rare or exotic orchid viruses?



Some come in other shapes as well

*Pterostylis blotch virus*

*Cucumber mosaic virus*

*Cymbidium ringspot virus*

*Cymbidium chlorotic mosaic virus*

*Capsicum chlorosis virus*

*etc*

# Virus mixtures

Unfortunately, mixed infections are quite common.

This can result in varied symptoms (resulting from the different viruses).

# What can we do about them?

"If you know the enemy and know yourself you need not fear the results of a hundred battles"

**Sun Tzu – The Art of War**

# Virus detection

Viruses cannot be seen using a microscope

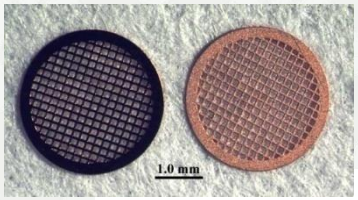
Symptoms are useful but unreliable

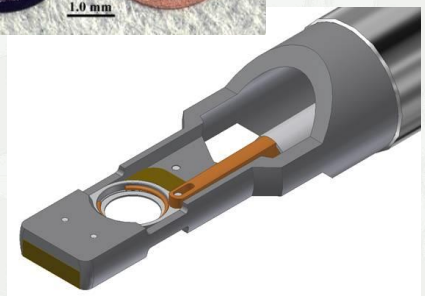
Symptoms can vary with:

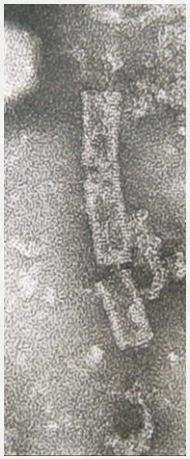
- mixed infections
- host species & cultivar
- virus strain
- age of plant at infection
- time since infection
- environmental conditions (esp. temperature)
- plant stress

Asymptomatic infections can also occur

# Electron microscopy

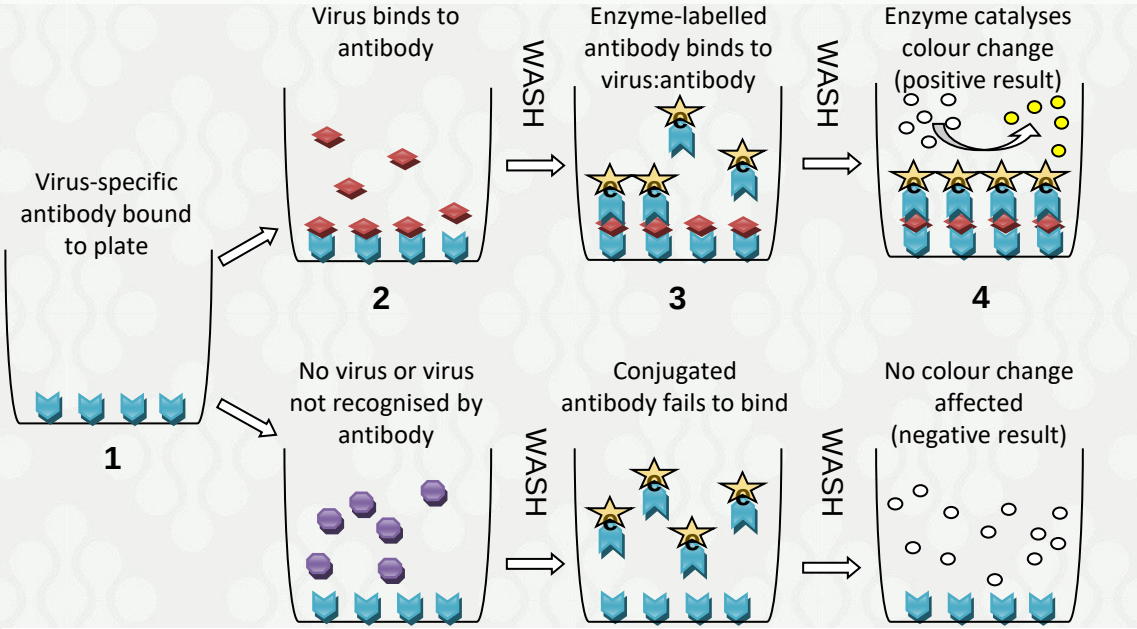


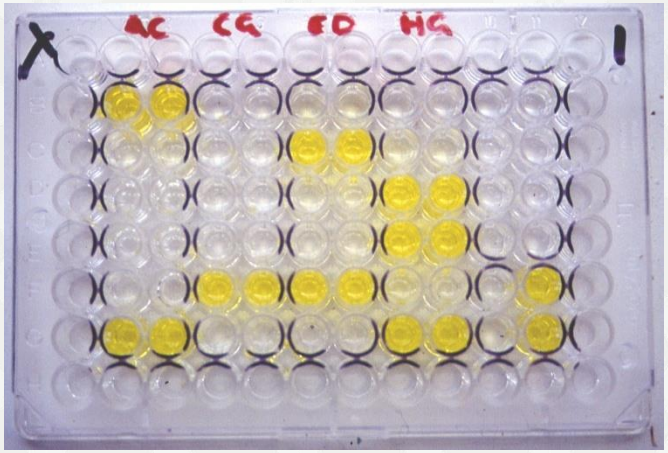





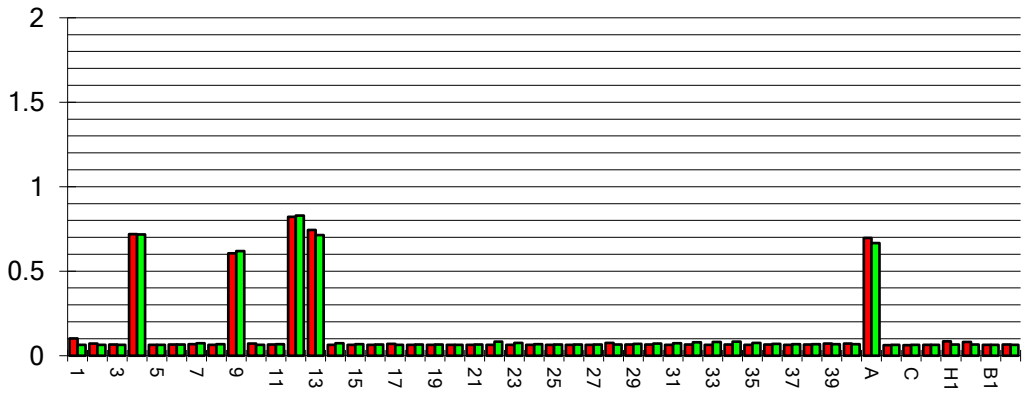
https://mifrah.com/electron-microscope/

# ELISA

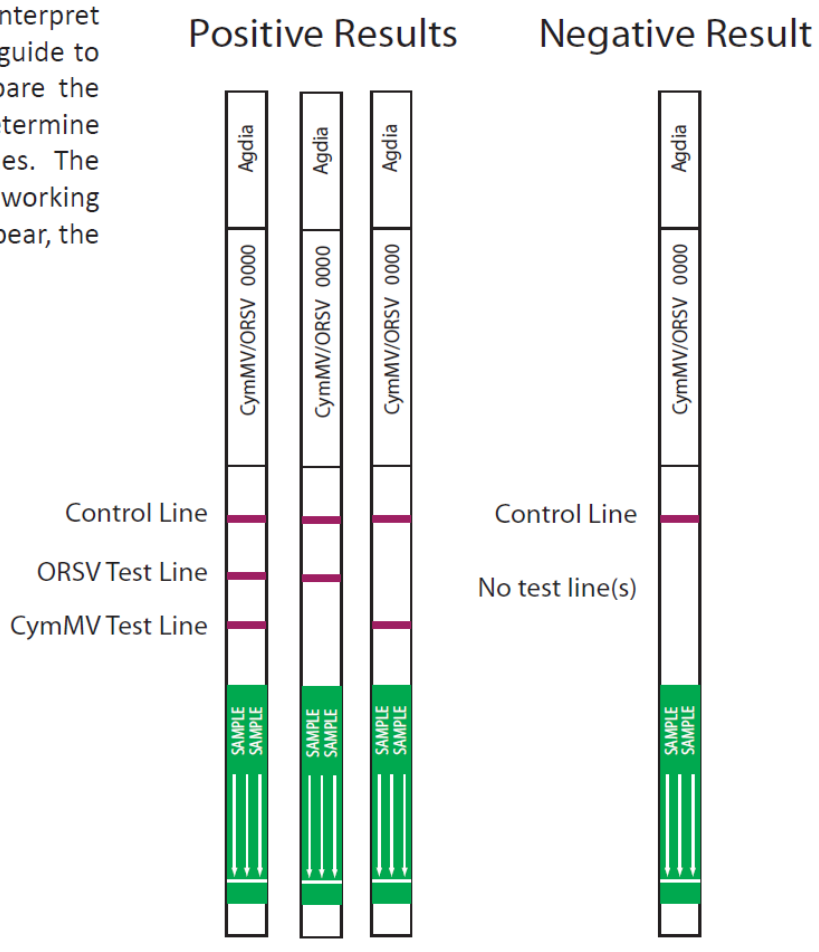




Wilson CR (2014) Applied Plant Virology, CABI



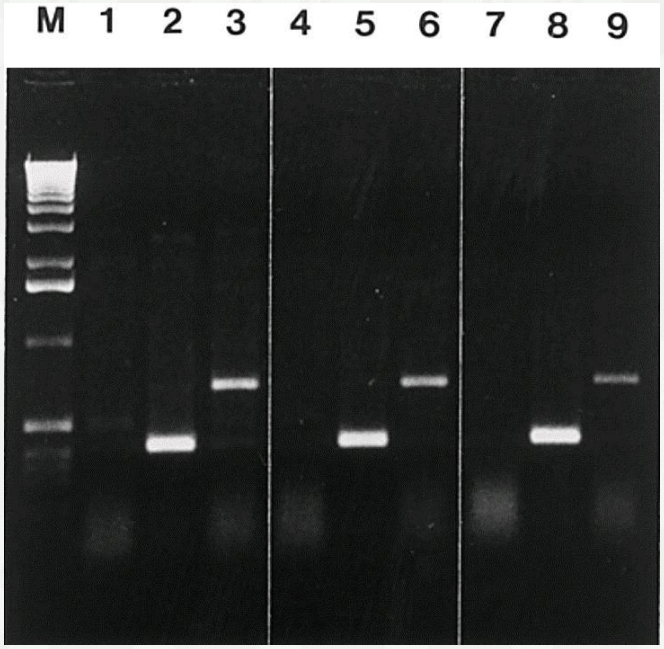
# Lateral flow test kits

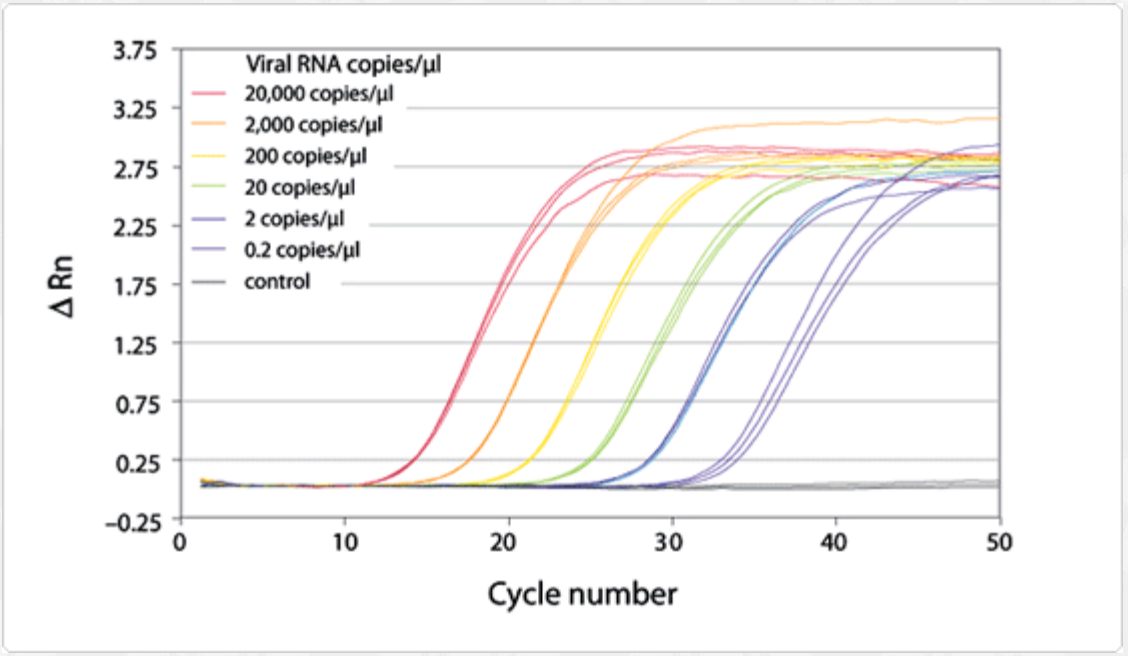
Remove test strip from extract and interpret results. Use the images provided as a guide to determine results. If necessary, compare the ImmunoStrip with the images to determine the positions of the test and control lines. The control line assures that the test is working properly. If the control line does not appear, the test is invalid.


Agdia User Guide: CyMV 7 ORSV ImmunoStrip

# Molecular tests (RT-PCR)

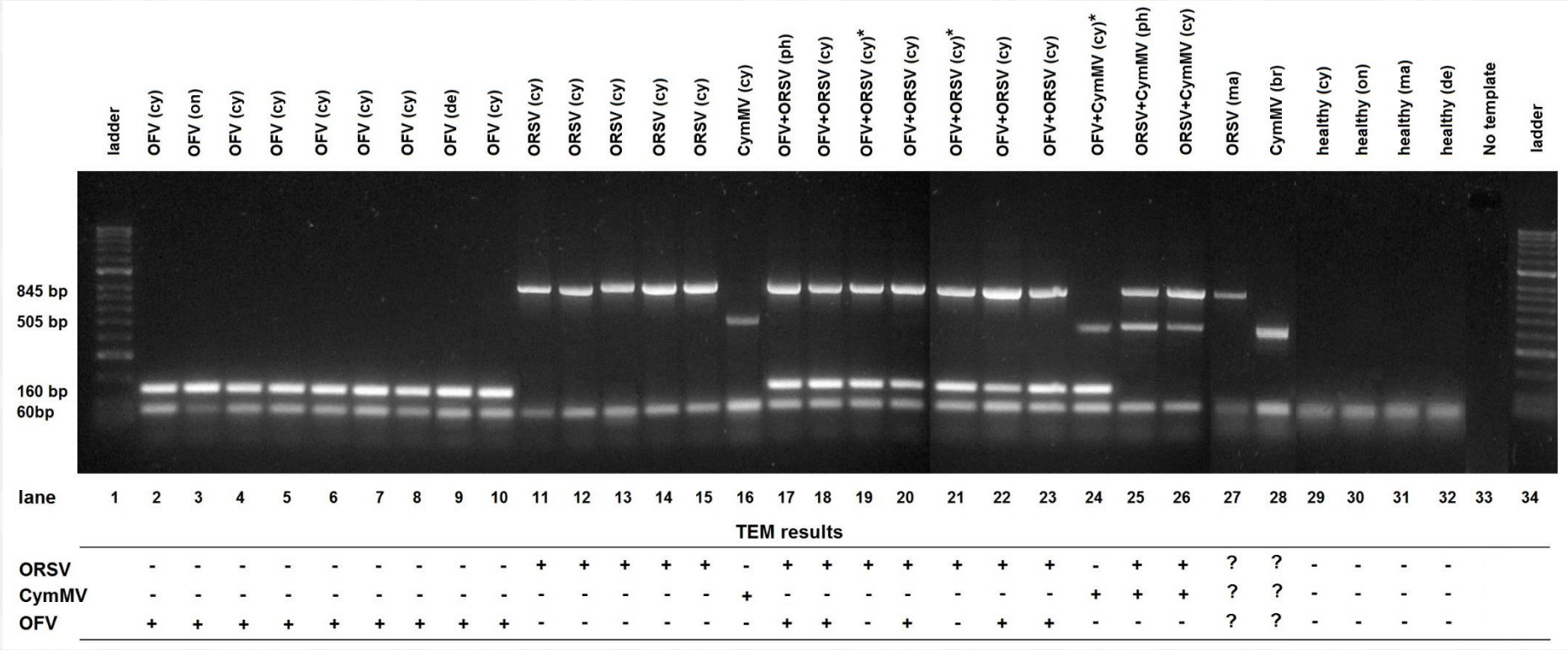



Detects DNA sequences specific to the target virus

qRT-PCR is extremely sensitive and can also tell you how much virus is present

# Multiplex (RT-PCR)




| lane | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **TEM results** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **ORSV** | | - | - | - | - | - | - | - | - | - | + | + | + | + | + | - | + | + | + | + | + | + | + | - | + | + | ? | ? | - | - | - | - | | |
| **CymMV** | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | + | + | + | ? | ? | - | - | - | - | | |
| **OFV** | | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | + | + | - | + | - | + | + | - | - | - | ? | ? | - | - | - | - | | |

Ray Ali developed a multiplex assay to detect all three common orchid viruses

Ali R et al (2014) Arch Virol 159:3095-9

# Which test ?

| Test Sensitivity | electron microscopy | ELISA / lateral flow | RT-PCR (gel) | qRT-PCR |
|---|---|---|---|---|

Roughly a 100-fold improvement in sensitivity with each step

You can detect OFV in mites using RT-PCR

# Which test ?

## Virus specificity of test:

### Electron microscopy

- not virus specific (relies on different shaped particles)

### ELISA / lateral flow / RT-PCR / qRT-PCR

- will detect what is tested for only
- you will need multiple tests for all major viruses
- or a multiplex test (several viruses detected simultaneously)

# TASAG-ELISA

Plant virus testing service for over 30 years

TASAG ELISA is the Australian distributor for Agdia products including the lateral flow test for CyMV & ORSV

NATA accredited, providing fee for service testing

- screening of orchids for CyMV, ORSV & OFV ($27.26 per sample)
- best suited to symptomatic material
- growers learn what constitutes ‘virus symptoms’
- electron microscopy is fine for testing propagative material but is more reliable where symptoms are present

# Management of orchid viruses

You cannot cure plants of virus infections

There are no chemical treatments that will eliminate viruses

Removing symptomatic leaves will not eliminate viruses

# Management of orchid viruses

## Avoid the problem (quarantine it)

- Be extra vigilant in what you bring into your collection or glasshouse (including tissue cultures).
- Always source from reputable sellers.
- Keep new material separate from your main collection and observe for any unusual symptoms until tested.
- Given virus infections can occasionally be symptomless, a virus test is good insurance.

# Management of orchid viruses

## Get rid of the problem (detect & destroy)

If you have virus symptoms present, or suspect a virus may be present:

- Conduct virus tests.
- Infected plants should be quickly removed & destroyed.
- Remember recent infections may not be obvious, so keep an eye on your collection & retest anything suspicious.
- For very valuable material you could try and recover from true seed but be careful.

# Management of orchid viruses

## Stop or slow the spread (keep it clean)

- Sterilise cutting tools (e.g., $Na_3PO_4$, alcohol, flame)
- Clean pots and benches (bleach)
- Wash hands regularly and use plenty of soap and water or use disposable gloves and change between plants
- Keep plants separated on benches
- Avoid excessive handling of plants

# Management of orchid viruses

## Avoid propagating the problem

- Do not propagate from infected plants.
  (test before using as mother plants).
- Take care when propagating using true seed as the material surrounding the seed may contain ORSV.
- Do not reuse media.
  (residual infected orchid material and/or mites could still be present).

# Management of orchid viruses

## Stop or slow the spread (stop the mites)

### Appropriate pesticide use

### Biological control

- *Phytoseiulus persimilis* and other predatory mites may feed on mites. It is not known whether this will give reliable control.

### Non-chemical control

- Regular sprays with water can remove some mites (but in citrus this encouraged virus spread so be wary)

# What don't we know

We need a better understanding of:

- Virus symptom expression and effect of temperature & host.
- Virus movement within host plants.
- Mite transmission dynamics & mite:virus association.
- Transmission during pollination & seed handling.
- OFV antibody production (?)

https://research.qut.edu.au/grc/research-programs/opportunities-for-students/

# Any Questions?

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