

# Diagnostic testing on non-symptomatic leaves

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## Key Question and Aim

Determine if *Pseudomonas syringae*. pv. *Actinidiae* (Psa) lives inside leaves without Psa symptoms or not in New Zealand.

### Outcome

- Psa (virulent and less-virulent) haplotypes were able to be isolated from the surface of symptomless leaves as they have an epiphytic growth habit.
- There was some evidence that Psa resides inside symptomless leaves, but before any conclusions are made these results need to be verified by further experimentation.
- For effective reduction of Psa-V inoculum in an infected orchard, vines at least 5m distant from leaves with symptoms need to be removed or treated with bactericides even if they do not show symptoms.
- These results suggest that Psa-LV is either being managed well by growers, or that it is not very active during January in New Zealand. However, Psa-V on two orchards from this study was very active when samples were taken.

## Methodology

Kiwifruit leaf tissue was sampled from 10 orchards in the Bay of Plenty. Samples were:

- Asymptomatic leaf tissue from an isolated uninfected orchard (control)
- Asymptomatic leaf tissue immediately adjacent to symptomatic leaf tissue on an infected orchard
- Asymptomatic leaf tissue 1m, 2m and 5m from symptomatic leaf tissue on the same cane on an infected orchard

Seven 1cm diameter leaf discs were cut out from every sample and were processed in one of three ways:

- Tissue was surface sterilised with 70 percent ethanol and hypochlorite and by rinsing with sterile deionised water (SDW) following the protocol of Everett et al. (2003) (3 discs)
- Tissue was not surface sterilised (3 discs). This protocol and sampling procedure will be repeated for kiwifruit cane tissue.
- Tissue was washed in 1ml bacterial saline by placing in a plastic bag and gently massaging.

A 100- $\mu$ l aliquot was spread over a Petri plate containing King's medium B (King et al. 1954) (1 disc). The discs from the leaf with symptoms were taken from tissue with leaf spots. Two surface sterilised and two non-surface sterilised discs were placed in an eppendorf tube and stored at -80C for future reference.

Isolations were made from the remaining two discs by crushing surface sterilised and non-surface sterilised tissue in 100  $\mu$ L SDW and spreading on a Petri plate containing King's medium B.

DNA from washings from Petri plates incubated at 25°C for 24 hours was extracted using the boiling method. DNA was tested by real-time Polymerase Chain Reaction (PCR) and the primers of Rees-George et al. (2010). Bacterial 23S primers (Rees-George et al. 2010) were used to check the quality of the DNA.

Results were reported as strong positive when the crossing threshold (CT) was <30 cycles, as weak positives when the CT was 30-35 cycles, and as negative when the CT was >35 cycles. The melting temperature was also used to confirm that the amplified product was Psa.

## Next Steps

- Canes now need to be sampled and tested from the same orchards, using the same sampling and sample preparation strategies.
- Further investigation of the survival time of Psa on leaf surfaces and whether it can reside inside symptomless leaves should be conducted.