

# Can Psa be detected in cane material?

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## Key Question and Aim

Determine whether *Pseudomonas syringae* pv. *Actinidiae* (Psa) can be detected in the cane tissues of vines showing secondary die-back symptoms.

### Outcome

- Preliminary data has indicated that Psa is present in canes of vines where die-back symptoms are observed.
- This suggests that canes need to be cut and removed when such symptoms exist to reduce the likely spread of the pathogen throughout the remaining vine.

## Methodology

Isolation of Psa from leaves:

1. Place kiwifruit leaf in an individual bag and add 10ml of sterile distilled water. Massage for 5 to 10 minutes.
2. Take out 100µl (micro litres) of washing suspension and streak on to a plate of King's B medium supplemented with cyclohexamide.
3. Incubate at 28°C for two days.
4. Sub-culture colonies that show Psa colony morphology.

Isolation of Psa from canes:

1. Surface sterilise cane pieces in 1 percent sodium hypochlorite for 3 minutes.
2. Rinse three times with sterile, distilled water.
3. Take cleaned cane pieces and using a sterile blade remove the bark, slicing off slivers of wood.
4. Place the slivers of tissue in a sterile Petri dish, add about 500µl of sterile distilled water and cut the slivers into smaller pieces.
5. Take 100µl of the suspension and streak on King's B medium supplemented with cyclohexamide.
6. Incubate at 28°C for two days.
7. Check plates for the presence of Psa by morphology and Polymerase Chain Reaction (PCR isolation of DNA and the PCR protocol were carried out as described in Rees-George et al. (2010) and Vanneste et al. (2010)).

## Next Steps

This shows that Psa can enter the kiwifruit vine and move rapidly in the canes. How far and how fast it can move will be determined in part using green fluorescent proteins (GFP) expressing derivatives of Psa. This work is in collaboration with Dr. Francesco Spinelli from Bologna University.

If the ability to cause secondary symptoms is a haplotype characteristic, then it would be important to determine in an orchard infected by that same haplotype whether Psa is found in cane tissues of vines showing only leaf spot symptoms.