

PLANT & FOOD RESEARCH *Pseudomonas syringae* pv. *actinidiae* (Psa) RESEARCH NOTE

PROJECT DETAILS

Project Title	<i>Testing for Psa contamination of surfaces sampled on December 2011 (1/3)</i>
Project Leader	<i>Kerry Everett (Shamini Pushparajah)</i>
Date (Month, Year)	<i>January 2012</i>

KEY QUESTION AND AIM

Does Psa inoculum lodge on clothing, tools and vehicles?

Aim: To identify key human/material vectors, to aid minimisation of orchard to orchard, and within-orchard, spread of Psa.

METHODOLOGY

1) Experimental Design

A Psa-V positive kiwifruit orchard c. 2 km from the centre of the Psa affected zone in Te Puke was chosen as the sample site. A number of samples were taken, with replication wherever possible, to determine the main sources of potential spread within an orchard. Samples were taken on 13 December 2011, when the weather was overcast with some showers. The rabbits were shot that night and sampled the next day. This is the first of three times when samples will be taken.

2) Methods and/or Protocols

Samples were taken with the aid of sterile cotton wool swabs that are used for sampling for bacteria in hospitals. After rubbing the cotton wool over the surface to be sampled, the swabs were replaced in their container and transported to the laboratory at Mt Albert Research Centre. Swabs were then washed for 1 minute in 1 ml sterile bacterial saline (BS) solution (0.75% NaCl), after which a 100- μ l aliquot was removed and spread with a sterilised bent glass rod on King's medium B (King et al. 1954) in a Petriplate. Petri plates were incubated at 28°C for 24-48 hours, washed with 1 ml of bacterial saline (BS), which was transferred to a sterile 1.5 ml Eppendorf tube, and the total volume adjusted to 1 ml. The bacterial suspension was vortexed and 100 μ l was aliquoted into 900 μ l of BS. Following centrifugation for 5 min at 8500 rpm, the resultant pellet was resuspended in 1 ml BS, centrifuged again then resuspended in 1 ml 1 mM EDTA. A 200- μ l aliquot of this suspension was then immersed in water at 100°C for 5 min, then placed immediately on ice. A 1- μ l aliquot of this suspension was used as a template in PCR reactions.

The PCR primers F3/R4 described in Rees-George et al. (2010) were used in real-time PCR. A dilution series from DNA previously extracted from the Psa type culture (ICMP 9617) was included as the positive control, and there was a negative water control.

Those surfaces that were sampled were:

1. People (5 replicates)
 - a. Hat/hair
 - b. Coat
 - c. Leggings
 - d. Gumboots (bottom surface)
 - e. Pouch (inside)
 - f. Work boots (bottom surface)
 - g. Hands/arms
2. Vehicles (7 replicates altogether)
 - a. Gator (2 replicates - one in the field and the other in the shed) and trailer in the field (1 replicate)
 - i. Accelerator
 - ii. Brake
 - iii. Seat
 - iv. Gear lever
 - v. Floor
 - vi. Steering wheel
 - vii. All four tyres
 - viii. Trailer gate behind vehicle
 - ix. Both trailer tyres
 - x. Trailer tray
 - b. Quad bike (2 replicates)
 - i. Right pedal
 - ii. Left pedal
 - iii. Handle bars
 - iv. Seat
 - v. All four tyres
 - c. Spray Tractor in the shed (1 replicate)
 - i. Steering wheel
 - ii. Gear lever
 - iii. All four tyres
 - d. 4x4 passenger vehicle (1 replicate)
 - i. Accelerator
 - ii. Brake
 - iii. Steering wheel
 - iv. Seat
 - v. All four tyres (before and after sterilising spray)

3. Tools

- a. Crowbar
- b. Hammer handle
- c. Toolbox handle
- d. Fencing tool handle
- e. Drill bit
- f. Secateurs (2 replicates)
- g. Shovel handle
- h. Spade
- i. Tip squeezer (2 replicates)

4. Rabbit feet (8 replicate rabbits)

5. Secateurs were sampled before and after using several different bactericides. The secateurs were contaminated with Psa by cutting through a brown cankered area on an infected kiwifruit cane (Figure 1). The bactericides that were tested were Varicide®, 70% ethanol, 1% Sporekill®, 4% Culticlean®. The bactericides were applied by a. spraying, b. dipping and c. soaking for 2 minutes. Secateurs were cleaned by spraying with 70% ethanol between samples, followed by wiping with a paper towel, and were then re-contaminated by cutting through the brown canker.



Figure 1: Canker on kiwifruit vine infected with Psa-V. Orange marked area is where secateur blades were inserted to contaminate them with Psa.

KEY RESULTS (all results must be auditable in terms of access to raw data if required)

1. People

a. There was one weak positive sample from a coat (Figure 2). All other samples were negative.



Hat	Green	Hair	Green	Hair	Green	Hat	Green	Hat	Green
Coat	Green	Coat	Green	Coat	Green	Coat	Green	Coat	Yellow
Leggings	Green	Leggings	Green	Leggings	Green	Leggings	Green	Leggings	Green
Gum boots	Green	Gum boots	Green	Gum boots	Green	Gum boots	Green	Gum boots	Green
Pouch	Green			Pouch	Green	Pouch	Green	Pouch	Green
Work boots	Green					Work boots	Green	Work boots	Green
		Arms	Green				Green	Inside coat	Green
Hands	Green	Hands	Green	Hands	Green	Hands	Green	Hands	Green

Figure 2: Results of Real-time PCR testing of bacteria grown from swabs taken from clothing and surfaces associated with orchard workers. Green is negative, yellow is a weak positive for Psa.

2. Vehicles

a. Gator and trailer

There were five strong positive samples from the Gator and the trailer in the field, and none from the Gator in the shed. Four of the positive samples were from tyres (two from the trailer and two from the Gator) and one positive was from the upright front of the trailer behind the Gator tyres (Figure 3).



Trailer	field
Tyre right	red
Tyre left	red
Tray	green
Upright behind Gator	red

Gator	field	shed
Accelerator	green	green
Brake	green	green
Seat drivers	green	green
Floor	green	green
Steering wheel	green	green
Tyre right front	red	green
Tyre left rear	red	green
Tyre left front	green	green
Tyre right rear	green	green

Figure 3: Results of Real-time PCR testing of bacteria grown from swabs taken from surfaces associated with a Gator in the field or stored in the shed. Green is negative, red is a strong positive for Psa.

b. Quad bike

There was one weak positive sample from one quad bike tyre (Figure 4). Both quad bikes were in the shed when sampled.



Quad bikes	Bike 1	Bike 2
Right pedal	Green	Green
Left pedal	Green	Green
Handle bars	Green	Green
Seat	Green	Green
Tyre right front	Yellow	Green
Tyre left front	Green	Green
Tyre right rear	Green	Green
Tyre left rear	Green	Green

Figure 4: Results of Real-time PCR testing of bacteria grown from swabs taken from surfaces associated with two quad bikes stored in the shed. Green is negative, yellow is a weak positive for Psa.

c. Spray tractor in the shed

There were no positive samples from the spray tractor that had been stored in the shed for 7 days since spraying the orchard (Table 1).

Table 1: Results of Real-time PCR testing of bacteria grown from swabs taken from surfaces associated with a spray tractor stored in the shed. Green is negative for Psa.

Spray tractor	shed
Right pedal	Green
Left pedal	Green
Steering wheel	Green
Gear lever	Green
Tyre right front	Green
Tyre left front	Green
Tyre right rear	Green
Tyre left rear	Green

d. 4x4 passenger vehicle

There was only one weak positive sample from a tyre after it had been through the disinfection spray (Table 2).

Table 2: Results of Real-time PCR testing of bacteria grown from swabs taken from surfaces associated with a 4x4 passenger vehicle after it had been driven on the orchard for 3 and 6 hours and after passing through a disinfection spray. Green is negative, yellow is weak positive for Psa.

4x4 passenger vehicle	3 hours	6 hours	after spray
Tyre right front	Green	Green	Green
Tyre left front	Green	Green	Green
Tyre right rear	Green	Green	Yellow
Tyre left rear	Green	Green	Green
Accelerator	White	Green	White
Brake	White	Green	White
Driver's seat	White	Green	White

3. Tools

All samples from tools were negative.

4. Rabbit feet

There were four strong positive samples and one weak positive sample from rabbit feet (Figure 5).

Rabbit	Block no.	Psa
1	1W	Red
2	B2	Yellow
3	B47	Red
4	14E	Red
5	30	Green
6	30	Green
7	30	Red
8	Random	Green

Figure 5: Results of Real-time PCR testing of bacteria grown from swabs taken from rabbit feet. Rabbits were shot the previous night and the blocks on which they were found are indicated. Green is negative, yellow is weak positive and red is a strong positive for Psa.

5. Secateurs before and after bactericides

The first time that the secateurs were used to cut through the canker on the infected kiwifruit vine successfully contaminated the secateurs (Table 3). Despite wiping with a paper towel after every application, residual 70% ethanol that was used between subsequent treatments killed all Psa on the secateurs.

Table 3: Results of Real-time PCR testing of bacteria grown from swabs taken from secateur blades before and after treatment with bactericides applied using three different methods. Green is negative, red is strong positive for Psa.

	Varicide®	70% ethanol	1% Sporekill®	4% Culticlean®
contaminated control	Red	Green	Green	Green
spray	Green	Green	Green	Green
dip	Green	Green	Green	Green
soak	Green	Green	Green	Green

FUTURE RESEARCH STEPS

- 1) Two more times for sampling are planned.
- 2) Samples need to be taken from a commercial orchard, concentrating on vehicles.
- 3) Dogs' paws need to be tested.

RECOMMENDATIONS FOR INDUSTRY

- 1) This study has shown that soil is a major source of inoculum and any activity that results in movement of soil could potentially spread the disease
- 2) Any surface on which soil adheres should be thoroughly cleaned and disinfected before transferring to another orchard, or to another block on the same orchard.

References

King EO, Ward MK, Raney DE 1954. Two simple media for the demonstration of pyocyanin and fluorescein *Journal of Laboratory and Clinical Medicine* 44: 301-307.

Rees-George J, Vanneste JL, Cornish DA, Pushparajah IPS, Yu J, Templeton MD, Everett KR 2010. Detection of *Pseudomonas syringae* pv. *actinidiae* using Polymerase Chain Reaction (PCR) primers based on the 16S-23S rDNA intertranscribed spacer region and comparison with PCR primers based on other gene regions. *Plant Pathology* 59: 453-464.

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This report has been prepared by The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research), which has its Head Office at 120 Mt Albert Rd, Mt Albert, Auckland.

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