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

PROJECT DETAILS

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

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-ul 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^{o}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 ul was aliquoted into 900 ul 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-ul aliquot of this suspension was then immersed in water at 100^{o}C for 5 min, then placed immediately on ice. A 1-ul 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	
Coat	
Leggings	
Gum boots	
Pouch	
Work boots	
Hands	

Hair	
Coat	
Leggings	
Gum boots	
Arms	
Hands	

Hair	
Coat	
Leggings	
Gum boots	
Pouch	
Hands	

Hat	
Coat	
Leggings	
Gum boots	
Pouch	
Work boots	
Hands	

Hat	
Coat	
Leggings	
Gum boots	
Pouch	
Work boots	
Inside coat	
Hands	

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	
Tyre left	
Tray	
Upright behind Gator	

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

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		
Left pedal		
Handle bars		
Seat		
Tyre right front		
Tyre left front		
Tyre right rear		
Tyre left rear		

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	
Left pedal	
Steering wheel	
Gear lever	
Tyre right front	
Tyre left front	
Tyre right rear	
Tyre left rear	

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			
Tyre left front			
Tyre right rear			
Tyre left rear			
Accelerator			
Brake			
Driver's seat			

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	
2	B2	
3	B47	
4	14E	
5	30	
6	30	
7	30	
8	Random	

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				
spray				
dip				
soak				

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 fluorescin 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.

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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