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

PROJECT DETAILS

Project Title	*Testing for Psa contamination of surfaces sampled on March 2012 (2/3)*
Project Leader	*Kerry Everett (Shamini Pushparajah, Michele Vergara, Ngaire Larsen)*
Date (Month, Year)	*May 2012*

KEY QUESTION AND AIM

Does Psa inoculum lodge on clothing, tools and vehicles?

Aim: To identify key human/material vectors, to help to minimise orchard to orchard, and within-orchard, spread of Psa

METHODOLOGY

1) Experimental Design

A Psa-V positive kiwifruit orchard c. 2 km, and a second site c. 20 km from the centre of the Psa affected zone in Te Puke, were chosen as the sample sites. The first orchard was diagnosed Psa-V positive in February 2011, and the second site in October 2011. A number of swabs were taken from different surfaces, with replication wherever possible, to determine the main sources of spread within an orchard. Samples were taken on 22 and 23 March 2012, when the weather was fine and sunny. Three of the people who were swabbed were picking fruit on the second infected 'Hort16A' orchard and had started at 0800 h that morning. They were sampled starting at 1300 h. Two tractors and trailers and one Gator® on this same orchard were also swabbed. One person, who was a PFR employee, was involved in monitoring heavily infected orchards, and had been on two orchards before samples were taken. He wore disposable suits with a head covering, used hand sanitiser between orchards, and disposable plastic coverings on his feet that were discarded and replaced after each orchard. The other two Gators and the quad bikes had been driven on the first infected orchard during normal orchard maintenance and were sampled immediately after they were returned to the shed at the end of the working day. Four dogs that had been running around for 2-3 hours before being sampled were from three different orchards that were infected with Psa-V. The dogs were sampled by swabbing their paws.

2) Methods and/or Protocols

Samples were taken using sterile cotton wool swabs of the type that are used for sampling for bacteria in hospitals. The swabs were dipped in sterile bacterial saline (BS) solution (0.85% NaCl) and then the cotton wool was rubbed 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 BS solution, 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 Petri plate. Petri plates were incubated at 28^{o}C for 24-48 hours, washed with 1 ml of 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) and the primers HopZ2b Psa-V described in Rikkerink et al. (2011) 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 swabbed were:

1. People (4 replicates)
    a. Hat/hair
    b. Coat/vest
    c. Gloves
    d. Footwear (bottom surface)
    e. Pouch (inside)
2. Vehicles (9 replicates altogether)
    a. Gator® (3 replicates)
        i. Accelerator
        ii. Brake
        iii. Seat
        iv. Gear lever
        v. Floor
        vi. Steering wheel
        vii. All four tyres
    b. Quad bike (2 replicates)
        i. Right pedal
        ii. Left pedal
        iii. Handle bars
        iv. Seat
        v. All four tyres
    c. Tractors in the field during picking (2 replicates)
        i. All four tyres
    d. Trailers in the field during picking (2 replicates)
        i. Both tyres
3. Dog's paws (4 replicate dogs)
4. Secateurs were sampled before and after using three different bactericides. The bactericides that were tested were Varicide®, 70% ethanol, and Trigene®. The secateurs were contaminated with Psa by cutting through a brown cankered area on an infected kiwifruit cane and were immediately swabbed. The bactericides were then applied by spraying, and secateurs were swabbed again. After the second swab, secateurs were used to cut through the infected canker five times, then swabbed, cut through a canker another five times, then swabbed and then used to make a further five cuts followed by swabbing. A total of 15 cuts were made before the final swabs were taken. A different pair of secateurs was used for each bactericide tested.

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

1. People (4 replicates)

When surfaces associated with people were swabbed, only one sample resulted in a weak positive test, and that was only with the F3/R4 primers (Table 1). This one positive sample was collected from the bottom of the disposable plastic foot cover from the PFR employee.

Table 1: Results of qPCR testing using F3/R4 Psa primers (Psa) and HopZ2b Psa-V primers. Persons no. 1-3 were pickers, and person no. 4 was a PFR employee who had been surveying infected orchards. Green is negative, yellow is weak positive, numbers are Cq values.

	Hat/hair		Coat/vest		Gloves		Footwear (bottom surface)		Pouch	
Primers / Person	Psa	Psa-V	Psa	Psa-V	Psa	Psa-V	Psa	Psa-V	Psa	Psa-V
1	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-
4 (PFR)	-	-	-	-	-	-	34.8	-	-	-

2. Vehicles

Of the swabs collected from the various vehicle parts, only two were weakly positive for Psa. These two samples were both from tyres, one from a Gator and one from a trailer. Both vehicles were on the second orchard that was being picked.

Table 2: Results of qPCR testing using F3/R4 Psa primers (Psa) and HopZ2b Psa-V primers of DNA extracted from bacteria growing from swabs taken from various parts of vehicles. Green is negative, yellow is weak positive. Numbers are Cq values. Horizontal lines are negative values for each replicate.

	Gator®		Quad bike		Tractors		Trailer	
Number of replicates	3		2		2		2	
Primers	Psa	Psa-V	Psa	Psa-V	Psa	Psa-V	Psa	Psa-V
Right front	- - -	- - -	- -	- -	- -	- -	- -	- -
Left front	- - -	- - -	- -	- -	- -	- -	32.9 -	- -
Right rear	- - -	- - -	- -	- -	- -	- -		
Left rear	34.9 - -	- - -	- -	- -	- -	- -		
Steering wheel	- - -	- - -	- -	- -	- -	- -		
Pedal	- - -	- - -	- -	- -	- -	- -		
Tray	- -	- -						
Seat			- -	- -	- -	- -		

3. Dog's paws (4 replicate dogs)

All negative.

4. Secateurs before and after bactericides

Cutting through brown staining on the infected kiwifruit cane successfully contaminated the secateurs with Psa prior to Varicide® treatment and to a lesser Cq value prior to the 70% ethanol treatment (Table 3). However, the brown section of the cane that was used to contaminate the secateurs immediately before the Trigene® treatment apparently did not contain bacterial cells. Spraying with both Varicide® and 70% ethanol successfully decontaminated the secateurs. When up to 15 further cuts following the Varicide® treatment were made through the brown staining, Psa did not recontaminate the secateurs. In contrast, only 5 cuts were needed to recontaminate the secateurs treated with 70% ethanol.

Table 3: Results of Real-time PCR testing using F3/R4 Psa primers (Psa) and HopZ2b Psa-V primers of bacteria grown from swabs taken from secateur blades before and after treatment with bactericides applied using three different methods. Green is negative, yellow is weak positive, and red is strong positive for Psa. Numbers are Cq values.

	Varicide®		70% ethanol		Trigene®	
Primers / Treatment	Psa	Psa-V	Psa	Psa-V	Psa	Psa-V
contaminated control	13.7	19.2	28.9	34.8	-	-
spray	-	-	-	-	-	-
5 cuts	-	-	32.0	-	-	-
10 cuts	-	-	29.0	34.9	-	-
15 cuts	-	-	27.5	33.3	-	-

FUTURE RESEARCH STEPS

1) One more sampling time is planned.
2) Samples should be taken during rain, and the same samples immediately beforehand in the dry.
3) Wet dogs' paws need to be tested.
4) The tests of effectiveness of bactericides to decontaminate secateurs need to be repeated, and further bactericides tested.

RECOMMENDATIONS FOR INDUSTRY

1) This study has shown that in dry conditions Psa is less prevalent on the clothes of orchard workers and on farm vehicles, but can still be found on surfaces that are contaminated with soil (tyres and the bottom of footwear)
2) The results indicate that Varicide® is a more persistent disinfectant than 70% ethanol
3) Soil on any surface to which soil adheres (vehicles, footwear and equipment) presents a risk and 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.

Rikkerink E, Andersen MT, Rees-George J, Cui W, J V, Templeton MD 2011. Development of a rapid tool for the molecular characterisation of Psa haplotypes. Plant and Food Research Report to Zespri Group Limited Ref. VI1256. SPTS No. 6361.

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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