***Pseudomonas syringae* pv. *actinidiae* on surfaces in the orchard**

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Abstract A kiwifruit orchard that had tested positive for virulent *Pseudomonas syringae* pv. *actinidiae* (Psa-V) ca 2 km from the incursion detected in November 2010 was sampled on 13 December 2011, when the weather was overcast with some showers. Surfaces on people (hands, soles of footwear, clothing), vehicles (tyres, seats, floors, steering wheels, accelerators, brakes and gear levers), cleaned tools (shovels, tip squeezers, various handles, drill bit) and rabbit feet were rubbed with sterile cotton wool medical swabs. After incubation in bacterial growth medium, DNA was extracted and samples tested in real-time PCR with primers that detect Psa. Vehicle tyres, the upright front of a trailer towed behind a vehicle, and rabbit feet were the surfaces most frequently contaminated with Psa. The surfaces contaminated with Psa were also those that were contaminated with soil, demonstrating the importance of removing soil from implements, vehicles and clothing on infected kiwifruit orchards before using liquid sanitisers.

Keywords bacteria, tools, people, rabbits, vehicles, contamination, soil.

INTRODUCTION

The bacterial kiwifruit pathogen, *Pseudomonas syringae* pv. *actinidiae* (Psa), was not known to be present in New Zealand until it was isolated from kiwifruit leaves and flowers in November 2010 (Everett et al. 2011). A delimiting study by MAF Biosecurity New Zealand and subsequent typing showed that there were two strains of Psa in New Zealand, which are now called Psa-LV (low virulent strain) and Psa-V (virulent strain) (Chapman et al. 2011). Nationwide sampling of spotted kiwifruit leaves showed Psa-LV to be widespread and thus has probably been present in New Zealand for a significant period of time, although probably less than 20 years (Park et al. 2011), but symptoms were not severe and were not noticed. Psa-V, which causes conspicuous leaf

spotting, flower wilting and browning, cane die-back, wilting, brown staining, cankers and death in as little as 4 weeks on susceptible cultivars such as *Actinidia chinensis* Planch var. *chinensis* 'Hort16A' (ZESPRI® GOLD), is a recent introduction to the Te Puke district of New Zealand. Since November 2010, this strain has spread from the original site where systemic symptoms were found to over 50% of ZESPRI® GOLD orchards in New Zealand.

Little is known about Psa-V spread and its capacity for survival on surfaces such as clothing, machinery, tools and animals. Epidemiological studies and fungicide spray trials were originally conducted in Japan in the late 1980s and early 1990s immediately after the discovery of Psa as a new organism (Serizawa et al. 1989; Takikawa et al.

1989; Serizawa & Ichikawa 1993a, b, c, d; Serizawa et al. 1994), but little additional information that is of practical use to growers during an incursion has been published since. This study is the first in a series of three investigations of the ability of *Psa*-V to survive on various surfaces and will provide evidence for which surfaces on an infected orchard require careful cleaning and sanitation.

MATERIALS AND METHODS

A *Psa*-V positive kiwifruit orchard ca 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. Samples were taken on 13 December 2011, when the weather was overcast with some showers.

Samples were taken with the aid of sterile cotton wool medical swabs (Cultiplast® swab CE0051). 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 min in 1 ml sterile bacterial saline (BS) solution (0.75% NaCl), after which a 100-µl aliquot was removed and spread with a sterilised bent glass rod on King's medium B (King et al. 1954) amended with 200 mg/litre cycloheximide (Sigma®, USA) in a Petri plate. Petri plates were incubated at 28°C for 24–48 h, 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 µl was aliquoted into 900 µl 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-µl aliquot of this suspension was immersed in water at 100°C for 5 min, then placed immediately on ice. A 1-µl aliquot of this suspension was used as a template in Polymerase Chain Reactions (PCR).

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, International Collection of Microorganisms from Plants, Landcare Research) was included as the positive control, and there was a negative

water control. A Ct (crossing threshold) value of <30 was considered a strong positive, 30–35 a weak positive, and >35 negative.

The surfaces sampled were people, vehicles, tools and rabbit feet. Five people were sampled, and depending on the clothing that they were wearing, swabs were taken from hats or hair, coats, leggings, the bottom surface of gumboots and/or workboots, the inside of a tool pouch, and from hands or arms. Three people had been working on the orchard for ca 6 hours and were fixing wire trellis on a block that had been heavily infected by *Psa*-V resulting in the removal of all the vines. The workboots were stored in their office and they were wearing gumboots, leggings and raincoats because it was raining. One person was working in the nursery, which included an uncovered shadehouse as well as glasshouses. Because it was raining that person was working under cover. The fifth person was a researcher who was walking around the infected orchard collecting samples and recording data, was wearing a raincoat, gumboots and leggings, and was swabbed at the end of the day's sampling. All people were sampled when and where they were working. Seven vehicles were sampled, comprising two John Deere Gator™ Utility Vehicles, one that was stored overnight in the shed and the other that was being used in the field, two quad bikes that had both been stored in the shed for a day, a spray tractor that had been stored in the shed for 7 days, and a 4×4 passenger vehicle that had been used to collect samples on the orchard. Samples from vehicles were taken from the accelerator, brake pedal, seat, gear lever, floor, steering wheel and tyres. Samples from quad bikes were from both pedals, handle bars, the seat and tyres. A trailer towed behind a Gator on the orchard was also sampled; samples were taken from the trailer gate behind the vehicle, both trailer tyres, and the trailer tray. Several tools were also sampled. These had all been cleaned according to best practice by the orchard workers (<http://www.kvh.org.nz/vdb/document/676>). This consisted of scrubbing the tools followed by disinfection with Varicide®. The tools sampled were a crowbar, hammer handle, toolbox handle, fencing tool handle, drill bit, secateurs (2 replicates), shovel

handle, spade and tip squeezer (2 replicates). The rabbits, which were from the same orchard, had been shot at night then placed in a coolstore at 0.5°C and sampled the next day. The feet of eight rabbits were sampled.

RESULTS

Of 32 samples taken from five people only one sample, taken from the outside of a raincoat of one of the workers fixing the trellis, was weakly positive for Psa. All other samples were negative (Table 1).

There were five strong and two weak Psa positives from a total of 58 swabs taken from seven vehicles (Table 2). These positive samples were taken from tyres and the upright on the front of the trailer towed behind the Gator in the field.

When the feet from eight rabbits were sampled, there were four strong and one weak Psa-positive results, and three Psa-negative results (Table 3).

No samples collected from any of the tools gave positive PCR results.

Table 3 *Pseudomonas syringae* pv. *actinidiae* (Psa) presence based on testing DNA extracted from bacteria isolated from cotton wool swabs that had been rubbed over the feet of eight rabbits.

Replicate	PCR Result
1	++ ¹
2	+
3	++
4	++
5	-
6	-
7	++
8	-

¹Results of Psa tests: - = negative, + = weak positive, ++ = strong positive.

Table 1 *Pseudomonas syringae* pv. *actinidiae* (Psa) presence based on testing DNA extracted from bacteria isolated from cotton wool swabs that had been rubbed over a variety of surfaces from five separate people.

Person	Hat/Hair	Coat	Leggings	Gumboots	Workboots	Pouch	Arms/hands
1	- ¹	-	-	-	-	-	-
2	-	-	-	-	n.s.a. ²	n.s.a.	-
3	-	-	-	-	n.s.a.	-	-
4	-	-	-	-	-	-	-
5	-	+	-	-	-	-	-

¹Results of Psa tests: - = negative, + = weak positive, ++ = strong positive.

²n.s.a. = no sample available.

Table 2 *Pseudomonas syringae* pv. *actinidiae* (Psa) presence based on testing DNA extracted from bacteria isolated from cotton wool swabs that had been rubbed over a variety of surfaces from seven vehicles.

Place on vehicle that was swabbed	Gator™ field	Gator™ shed	Gator trailer	Quad bike 1	Quad bike 2	Spray tractor	4x4 vehicle
Accelerator/lever	- ¹	-	n.s.a. ²	-	-	-	-
Brake/pedal	-	-	n.s.a.	-	-	-	-
Seat	-	-	n.s.a.	-	-	-	-
Floor	-	-	-	-	-	-	-
Steering wheel/ handle bars	-	-	n.s.a.	-	-	-	-
Trailer upright	n.s.a.	n.s.a.	++	n.s.a.	n.s.a.	n.s.a.	n.s.a.
Tyres	2/4 ³	0/4	2/2	1/4	0/4	0/4	1/4
	++(×2)		++(×2)	+(×1)			+(×1)

¹Results of Psa tests: - = negative, + = weak positive, ++ = strong positive.

²n.s.a. = no sample available.

³The first value is the number of positive samples out of the total number of tyres tested (2 or 4 per vehicle). The strength of the positive response is indicated in the following row.

DISCUSSION

Rabbits' feet, tyres and the upright on the trailer towed behind the Gator all tested positive for Psa, and all were contaminated by soil. The trailer upright was covered with mud splashed by the Gator tyres. Several other pathovars of *Pseudomonas syringae* have been shown to be able to survive in moist soil for sufficiently long periods of time to ensure survival from season to season. These pathovars include pv. *glycinea* (Park & Lim 1985), pv. *tomato* (Goode & Sasser 1980) and pv. *lachrymans* (Kritzman & Zutra 1983). It is therefore not surprising that Psa could be found on surfaces contaminated with soil. However, in dry soil, in soil without plant material, or when bacterial cells were in a planktonic stage, survival by *P. syringae* in soil is reduced. For example, cells of *P. syringae* sprayed onto an orchard became undetectable in soil after 2 days (Lindow et al. 1988).

Psa was not as frequently retrieved from dried soil on the tyres of vehicles that had been stored in the shed as from moist soil from tyres in the field. This suggests that Psa was not surviving in dry soil, a behaviour similar to pv. *lachrymans* (Kritzman & Zutra 1983), but dissimilar to pv. *glycinea*, which survived in soil better when conditions were cold and dry (Park & Lim 1985).

The lack of positive detections from the soles of gumboots and workboots was unexpected. However, none of the surfaces were obviously contaminated by soil. The footwear was either being worn on concrete inside a glasshouse facility, or the soles were cleaned by walking through slightly damp uncut grass. The workboots had been stored in the office after disinfecting and cleaning. The consequent lack of contaminating soil on the bottom of the sampled boots would explain these negative results. The lack of isolations from soles of workboots stored in the office also supports the finding from vehicle tyres that Psa may not survive in dry soil, but could also be explained by diligent use of the footbath containing a disinfectant after leaving the orchard. In contrast, rabbit's feet were obviously contaminated with soil, and were also contaminated with Psa.

Reports of vertebrate vectors of a plant disease are rare, although *Erwinia amylovora* (the cause of fire blight of pear and apple trees) has been reported from bird feet (Seidel et al. 1994). Rabbits vectoring a bacterial disease of plants has not been previously reported. Insect vectors for *Pseudomonads* are relatively commonly reported (Everett 2011). However, bacterial diseases are spread by wind and rain, and extreme events such as hurricanes facilitate rapid and distant spread. As an example, despite 50 years of successful eradication of citrus canker hotspots in Florida, further attempts were abandoned after several hurricanes occurred in the same season during 2004 and 2005 (Gottwald & Irey 2007). Rabbits are not likely to be able to spread bacterial diseases as rapidly or as far as wind and rain, and may become contaminated as a consequence of the epidemic rather than making an important contribution to its spread. However, removal of pests from an orchard is good practice, and removal of this potential means of spread of Psa-V within an orchard may be beneficial.

It was reassuring that it was difficult to find Psa on the external surfaces of people, even though the conditions were ideal for spreading bacterial inoculum (light rain). Rain has consistently preceded disease outbreaks for several foliar blights caused by pathovars of *P. syringae*. The importance of rain in the epidemiology of these diseases has been recognised for decades. Rain disperses inoculum by splashing from leaf to leaf and from plant to plant, but this is only local dispersal (a few metres). However, several factors probably contributed to the lack of Psa detected in the current study: vines had already been removed from the block in which the orchard workers were working, light rain may not easily facilitate distant spread or create splash from contaminated soil, and because it had been raining for several hours before sampling, available inoculum may already have been dispersed from oozing cankers from vines on adjacent blocks. Gottwald & Irey (2007) state that the maximum concentration of citrus canker inoculum is released in the first 1–2 h after a period of rain begins, and after that there is a significant decline in inoculum concentration.

Further sampling from workers from blocks with infected vines would help to explain this result, and from workers on infected blocks during heavy rain and strong winds.

It was also reassuring that there was no Psa found on any of the tools that were sampled. The practices that were used on this orchard (cleaning followed by disinfection with Varicide®) were effective.

Those surfaces that were contaminated with Psa were also those that were contaminated with soil, indicating that soil may be as important as rain and wind for spreading Psa, especially if it stays moist. Of particular concern would be soil remaining on parts of vehicles that are normally not cleaned, such as wheelbases. Driving through a disinfectant bath would not remove contaminated soil and vehicles could remain potential carriers of Psa for several days, allowing large distances to be travelled. Thorough cleaning followed by disinfection would be required before vehicles from infected orchards should be allowed onto clean orchards.

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