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Pseudomonas syringae pv. *actinidiae* wound entry sites – cicada egg nests

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Executive summary

Pseudomonas syringae pv. *actinidiae* wound entry sites – cicada egg nests

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A number of kiwifruit management practices (girdling, pruning) create wounds that are potential infection sites for *Pseudomonas syringae* pv. *actinidiae* (Psa, bacterial canker of kiwifruit). The importance of wounds created by insects, in particular cicada egg-nest sites, as Psa infection ports, remains to be determined.

In this study, cicada egg-nests and artificial wounds (cuts) in canes of 'Hort16A' and 'Hayward' kiwifruit were inoculated to determine their potential role in infection by Psa. It was found that Psa can enter canes of the *Actinidia* cultivars 'Hort16A' and 'Hayward' through cicada egg-nest wounds. No significant difference in recovery of Psa was found between the cultivars.

There was a higher rate of recovery of Psa from inoculated cuts than from inoculated egg-nests irrespective of their ages. There was no significant difference in recovery of Psa between egg-nests of different ages.

Because of the limited number of samples and the differing numbers of samples in each treatment, the data set had large error values. Nevertheless, this preliminary trial has confirmed that cicada egg-nests do present an infection port for Psa. The extent of the risk that they represent has yet to be determined.

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1 Background

A number of kiwifruit management practices (girdling, pruning) create wounds that are potential infection sites for *Pseudomonas syringae* pv. *actinidiae* (Psa, bacterial canker of kiwifruit). Various research programmes are currently investigating canopy management systems to reduce pruning, the susceptibility of girdle wounds to infection, and technologies to protect wounds (VI1257). The importance of wounds created by insects, in particular cicada egg-nest sites, as Psa infection ports, remains to be determined.

Two endemic species of cicada, clapping cicada (*Amphipsalta cingulata*) and chorus cicada (*A. zelandica*), are pests of kiwifruit. Clapping cicadas are most common in coastal and new kiwifruit orchards and otherwise tend to occur at low densities. Chorus cicada is the more abundant of the two species, particularly at Te Puke and Katikati, where orchards are at high density, and can be considered the major cicada pest (Logan et al. 2011).

Cicadas can cause economic losses for growers in several ways. Sooty moulds associated with feeding by adult cicadas can lead to increased fruit rejection rates. Egg-laying by cicadas causes cane damage and often cane loss. Consequently, there may be gaps in the canopy or growers may be forced to select inferior quality replacement canes. Psa infection of vines via cicada egg-nests by Psa may be a further reason to consider cicada control.

In this study, cicada egg-nests in canes of 'Hort16A' and 'Hayward' kiwifruit were inoculated to determine their potential role in infection by Psa.

2 Methods

Two orchards of 'Hort16A' and 'Hayward' that had a history of cicada problems and that were considered to be free of Psa-V were identified in the Katikati-Waihi area. Each orchard was visited once or twice weekly between mid-January and mid-February 2012, the main egg-laying period for Chorus cicada. Recently laid egg-nests were marked at each visit. On 16 February, cane sections with marked egg-nests were removed to Te Puke Research Orchard (TPRO), pruned to c. 40 cm in length and classed by age of nest into three ('Hort16A') or four ('Hayward') groups. Cane sections segregated by nest age were further assigned in equal numbers without conscious bias to each of three dose treatment groups. A further group of cane sections without egg-nests was assigned to receive artificial wounding.

Egg nests were inoculated with Psa the day after harvesting. In order to facilitate recovery and identification, a Psa strain that is resistant to high concentrations of the antibiotic rifampicin was used. The inoculum was suspended in bacteriological saline (0.85% NaCl in sterile distilled water) at a rate of approximately 2×10^3 or 2×10^6 colony forming units per mL (cfu/mL).

2.1 Psa strain

Spontaneous rifampicin-resistant mutants of Psa were previously acquired by streaking isolates onto King's B medium (King et al. 1954) amended with 50 ppm rifampicin. Colonies that grew normally on this medium were then transferred to plates containing increasing concentrations of rifampicin (100, 150 and 200 ppm). The mutants were maintained on King's B media containing 100 ppm of rifampicin. The rifampicin-resistant mutant used in this trial (Psa 3.2.3/rif) was derived from Psa isolate 3.2.3, and was tested to ensure similar pathogenicity in leaf disc assays (data not shown). Psa 3.2.3 was isolated from kiwifruit leaf spots at Te Puke Research Orchard in February 2011 and was previously determined to be haplotype NZ-V.

2.2 Treatments

There were 26 treatments and 4-10 egg nests (replicates) per treatment. In each inoculated treatment, 50 µL of the inoculum suspension was pipetted into the egg nest wound. In the bacteriological saline (BS) controls, 50 µL of BS was used. In the 0 week (cut stem) treatments, BS or Psa was applied directly to a diagonal cut made in the cane with a sterile scalpel. The treatment schedule is shown in Table 1.

Table 1. Schedule of *Pseudomonas syringae* pv. *actinidiae* (Psa) treatments to cicada egg-nests.

trt #	Trt name	Cultivar	Egg nest age (weeks)
1	BS* control	'Hort16A'	0 (cut stem)
2	BS* control	'Hort16A'	1
3	BS* control	'Hort16A'	2
4	BS* control	'Hort16A'	3
5	BS* control	'Hayward'	0 (cut stem)
6	BS* control	'Hayward'	1
7	BS* control	'Hayward'	2
8	BS* control	'Hayward'	3
9	Psa 10^3 cfu/mL	'Hort16A'	0 (cut stem)
10	Psa 10^3 cfu/mL	'Hort16A'	1
11	Psa 10^3 cfu/mL	'Hort16A'	2
12	Psa 10^3 cfu/mL	'Hort16A'	3
13	Psa 10^3 cfu/mL	'Hayward'	0 (cut stem)
14	Psa 10^3 cfu/mL	'Hayward'	1
15	Psa 10^3 cfu/mL	'Hayward'	2
16	Psa 10^3 cfu/mL	'Hayward'	3
17	Psa 10^3 cfu/mL	'Hayward'	4
18	Psa 10^6 cfu/mL	'Hort16A'	0 (cut stem)
19	Psa 10^6 cfu/mL	'Hort16A'	1
20	Psa 10^6 cfu/mL	'Hort16A'	2
21	Psa 10^6 cfu/mL	'Hort16A'	3
22	Psa 10^6 cfu/mL	'Hayward'	0 (cut stem)
23	Psa 10^6 cfu/mL	'Hayward'	1
24	Psa 10^6 cfu/mL	'Hayward'	2
25	Psa 10^6 cfu/mL	'Hayward'	3
26	Psa 10^6 cfu/mL	'Hayward'	4

*BS – bacteriological saline

2.3 Isolations

Isolations were made from surface-sterilised cicada nests three weeks after inoculation as follows: Pieces of plant tissue (3 mm cross-sections of cane from the inoculation site) were aseptically excised and macerated in 2 mL BS, and left for at least five minutes. A 100 µL aliquot of the resulting suspension was then spread onto Kings B medium containing 100 ppm of rifampicin, which allowed the rifampicin-resistant strain to grow while restricting the growth of non-target bacteria and fungi. Plates were incubated at room temperature (c. 20°C), marked as Psa present/absent after three days, and re-checked after five days.

Additional isolations were made from treatments 19 and 23 (one-week-old cicada egg-nests of 'Hort16A' and 'Hayward', respectively, inoculated with the highest rate of Psa). For these treatments, isolations were also made from 2 cm either side of the inoculation site.

2.4 qPCR

DNA extractions were done on isolations that were initially marked as Psa 'unclear', and on a selection of those marked Psa-present. DNA was stored at -20°C until used for qPCR.

Subsequent identification used the method of Rees-George et al. (2010), modified for use with qPCR.

3 Results

The raw data are shown in Appendix 1. The statistical analyses (SAS, GENMOD procedure) of the principal factors (Psa inoculum rate, cultivar and egg nest age) are shown in Table 2.

Table 2. Logit means, standard errors and back-transformed means (% egg nests from which Psa was recovered).

Factor/Level	**Logit**		**Back-transformed means** (% egg nests Psa-positive)
	mean	**SE**	
Psa inoculum rate			
Psa High	**-0.2626**	**0.3304**	43.5
Psa Low	**-1.6258**	**0.4121**	16.4
(P-value)	***(0.0179)***		
Cultivar			
'Hort16A'	**-0.8440**	**0.3636**	30.1
'Hayward'	**-1.0443**	**0.3675**	26.0
(P-value)	***(0.6862)***		
Egg-nest age			
Cut	**0.5469**	**0.4097**	63.3
1	**-1.2177**	**0.4540**	22.8
2	**-1.2288**	**0.4792**	22.6
3	**-1.8770**	**0.7623**	13.3
(P-value)	***(0.0217)***		

In this experiment, no significant difference in Psa infection was found between cultivars. There was a significantly better recovery of Psa at the higher inoculum rate (10^6 cfu/mL compared with 10^3 cfu/mL). There was also significantly better recovery of Psa from the cuts compared with recovery from egg-nests of all ages. There was no significant difference in recovery of Psa from egg-nests of different ages.

Figure 1 and Figure 2 show the relationships between the ages of cicada egg-nests at inoculation, inoculum rate and % isolations testing positive for Psa ('Hort16A' and 'Hayward'). No Psa was recovered from four-week-old cicada egg-nests on 'Hayward'.

The additional isolations that were made from treatments 19 and 23 (one-week-old cicada egg-nests on 'Hort16A' and 'Hayward', respectively, inoculated with the highest rate of Psa) showed that there had been no, or minimal, movement of Psa from the point of inoculation. Psa was not found from any of the isolations made from 2 cm either side of the initial inoculation sites.

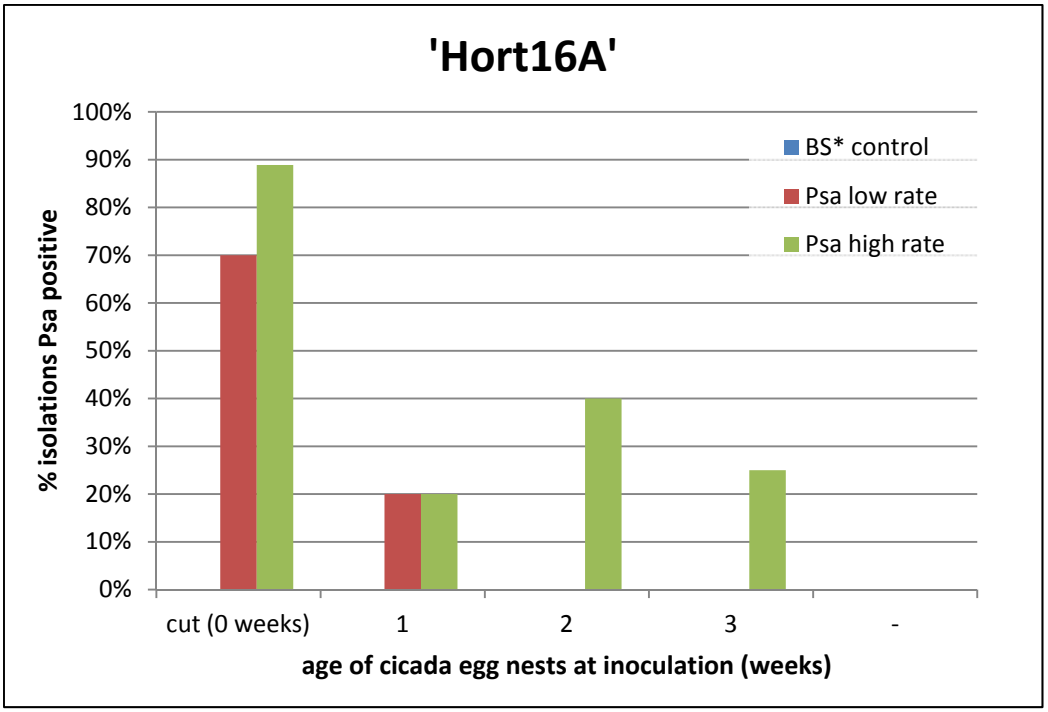

Figure 1. Relationship between the age of cicada egg-nests at inoculation, inoculum rate and % isolations testing positive for *Pseudomonas syringae* pv. *actinidiae* (Psa) (on 'Hort16A').

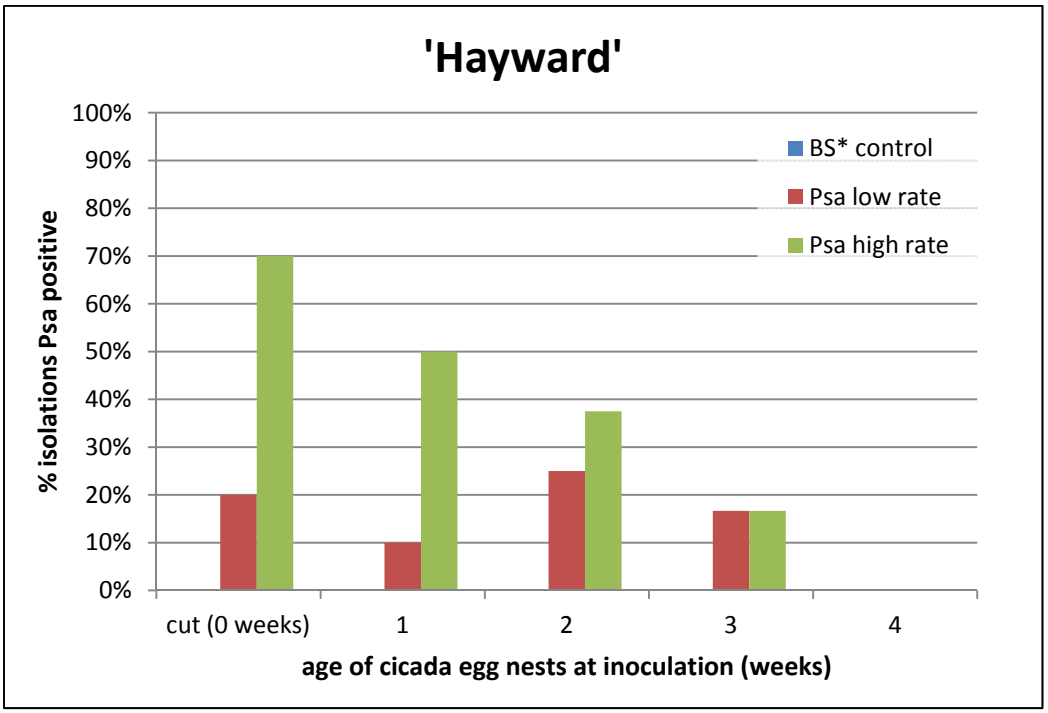

Figure 2. Relationship between the age of cicada egg-nests at inoculation, inoculum rate and % isolations testing positive for *Pseudomonas syringae* pv. *actinidiae* (Psa) (on 'Hayward').

4 Discussion

Pseudomonas syringae pv. *actinidiae* is thought to invade kiwifruit leaves through hydathodes, stomata and wounds (Serizawa & Ichikawa 1993). Other entry points for the bacterium are thought to include pruning wounds, fruit and leaf scars, as well as wind, hail and frost damage (Balestra 2011). In New Zealand, Psa has also been found to invade vines through girdling wounds (Snelgar et al. unpub.).

This work has found that Psa can also enter canes of the *Actinidia* cultivars 'Hort16A' and 'Hayward' through cicada egg-nest wounds. No significant difference in recovery was found between the cultivars.

The higher inoculum rate resulted in more infection; indicating that there may be a need for a threshold population of Psa to initiate infection. This has been shown for other phytopathogenic *Pseudomonas syringae* cultivars, such as *P. s.* cv. *syringae* on sweet cherry (Latorre et al. 2002). There had been no, or minimal, movement of Psa from the point of inoculation; however, the canes were cut lengths and were not actively growing and this may not be the case in entire plants.

There was a significantly higher rate of recovery of Psa from inoculated cuts than from inoculated egg-nests irrespective of their ages. There was no significant difference in recovery of Psa between egg-nests of different ages.

Because of the limited number of samples and the differing numbers of samples in each treatment, the data set had large error values. There was a suggestion of an age effect in the 'Hayward' high inoculum rate where there was less recovery from older egg-nests (not significant), and there was no recovery of Psa from 4-week-old egg-nests from 'Hayward'. A more detailed study will be needed to determine whether the observed trend in relation to nest age is real. Nevertheless, this preliminary trial has confirmed that cicada egg-nests do present an infection port for Psa. The extent of the risk that they represent has yet to be determined.

5 Acknowledgements

Thanks to Nihal DeSilva for statistical analysis of the data and Margaret Smith of Aongatete Coolstores Ltd for identifying Psa-free orchard blocks with a history of cicada problems and allowing regular entry for the tagging and removal of egg-nests.

6 References

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7 Appendix

Raw data

			replicates (cicada egg nests)									
Trt name	**Cultivar**	**Egg nest age (weeks)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
BS* control	'Hort16A'	0 (cut)	0*	0	0	0	0	0	0	0	0	0
BS* control	'Hort16A'	1	0	0	0	0	0	0	0	0	0	0
BS* control	'Hort16A'	2	0	0	0	0	0	0	0	0	0	0
BS* control	'Hort16A'	3	0	0	0	0	0					
BS* control	Hayward	0 (cut)	0	0	0	0	0	0	0	0	0	0
BS* control	Hayward	1	0	0	0	0	0	0	0	0	0	0
BS* control	Hayward	2	0	0	0	0	0		0	0		
BS* control	Hayward	3	0	0	0	0	0	0				
BS* control	Hayward	4	0	0	0	0	0					
Psa 10^3	'Hort16A'	0 (cut)	1	1	0	0	1	1	1	0	1	1
Psa 10^3	'Hort16A'	1	0	0	0	0	1	1	0	0	0	0
Psa 10^3	'Hort16A'	2	0	0	0	0	0	0	0	0	0	0
Psa 10^3	'Hort16A'	3	0	0	0	0						
Psa 10^3	Hayward	0 (cut)	0	0	0	1	0	0	1	0	0	0
Psa 10^3	Hayward	1	0	0	0	0	1	0	0	0	0	0
Psa 10^3	Hayward	2	1	1	0	0	0	0	0	0		
Psa 10^3	Hayward	3	0	1	0	0	0	0				
Psa 10^3	Hayward	4	0	0	0	0						
Psa 10^6	'Hort16A'	0 (cut)	1	1	1	1		1	1	0	1	1
Psa 10^6	'Hort16A'	1	1	0	0	0	0	0	1	0	0	0
Psa 10^6	'Hort16A'	2	0	1	0	1	0	1	0	0	1	0
Psa 10^6	'Hort16A'	3	0	1	0	0						
Psa 10^6	Hayward	0 (cut)	1	1	1	1	1	0	0	1	1	0
Psa 10^6	Hayward	1	0	1	1	1	1	0	1	0	0	0
Psa 10^6	Hayward	2	1	0	0	0	0	0	1	1		
Psa 10^6	Hayward	3	0	0	0	1	0	0				
Psa 10^6	Hayward	4	0	0	0	0	0					

*0 = Psa not present, 1 = Psa present

**results marked in yellow were confirmed by qPCR