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Pseudomonas syringae pv. *actinidiae* wound entry sites – cicada egg nest field trial

Tyson J, Curtis C, Dobson S, Logan D, Manning M, Rowe C

June 2012

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ZESPRI Group Limited

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Executive summary

Pseudomonas syringae pv. *actinidiae* wound entry sites – cicada egg nest field trial

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A number of kiwifruit management practices, particularly girdling and 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 entry sites, remains to be determined.

This study follows previous laboratory inoculation work that showed cicada egg-nests are a potential entry site for Psa infection. In this study, Psa infection of cicada egg-nests in canes of *Actinidia* cultivars 'Hort16A' and 'Hayward' was compared with adjacent, non-wounded canes to determine the potential role of egg-nest wounds in infection by Psa.

In two out of six kiwifruit blocks, one 'Hort16A' and one 'Hayward', Psa was more likely to be isolated from canes with egg-nests than canes without egg-nests ($P< 0.10$). When the three blocks of each cultivar were combined, Psa was more likely to be isolated from canes with egg-nests than canes without egg-nests for both 'Hort16A' ($P= 0.051$) and for 'Hayward' ($P= 0.025$).

Any wound is a potential entry point for Psa. Protection of wounds with copper and other ameliorants and the minimisation of wounding by control of cicadas are important considerations for disease management.

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1 Background

A number of kiwifruit management practices, particularly girdling and pruning create wounds that are potential infection sites for *Pseudomonas syringae* pv. *actinidiae* (Psa, bacterial canker of kiwifruit). Various research programmes have investigated, or are currently investigating, canopy management systems to reduce pruning, the susceptibility of girdle wounds to infection (Snelgar et al. 2012a; Snelgar et al. 2012b), and technologies to protect wounds. 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, Psa infection of cicada egg-nests in canes of *Actinidia* cultivars 'Hort16A' and 'Hayward' was compared with adjacent, non-wounded canes to determine the potential role of egg-nest wounds in infection by Psa.

This study follows a previous laboratory study (Tyson et al. 2012) that showed, through artificial inoculation, that cicada egg-nests are potential entry sites for Psa.

2 Methods

Three orchard blocks of ‘Hort16A’ with symptoms of Psa-V infection and three ‘Hayward’ blocks adjacent or previously adjacent to symptomatic ‘Hort16A’ blocks were sampled (Table 1).

Table 1. Symptoms of infection by *Pseudomonas syringae* pv. *actinidiae* for sampled kiwifruit blocks.

Orchard block	cultivar	Observations of Psa infection
1	‘Hort16A’	Heavy infection: Secondary symptoms widespread (shoot die-back and fresh ooze easy to find); removal of canopy due to infection by Psa-V imminent.
2	‘Hort16A’	Heavy infection: Secondary symptoms widespread (shoot die-back and fresh ooze easy to find).
3	‘Hort16A’	Low-medium infection: some shoot die-back and ooze.
4	‘Hayward’	Leaf-spotting present; adjacent to ‘Hort16A’ blocks that had canopy removed because of Psa-V infection in 2011.
5	‘Hayward’	Leaf-spotting present; adjacent to orchard block 1.
6	‘Hayward’	Leaf-spotting present; previously adjacent to three rows of ‘Hort16A’ removed because of Psa-V infection in 2011.

On 17 May 2012, two cane sections were removed from each of 25 vines within each block. The first cane section of each pair (approximately 10 cm in length) had a cicada egg-nest; the second cane section was taken from a similar position in the nearest adjacent unwounded cane (control cane).

All cane sections were sent to a Physical Containment (PC1+) laboratory at Plant & Food Research, Mt Albert Research Centre, Auckland, where isolations were made.

2.1 Isolations

A total of 300 isolations were made from the cane sections as follows:

Cane sections were surface sterilised by immersion in 95% ethanol for 1 minute. Isolations were made from the centre of the egg-nests and the corresponding areas on the paired, unwounded canes. Pieces of plant tissue (1-2 mm cross-sections of cane) were aseptically excised, macerated in 200 µL bacteriological saline (BS, 0.85% NaCl in sterile distilled water), and left for at least five minutes. A 100 µL aliquot of the resulting suspension was then spread across a semi-selective agar medium based on the ‘KBC’ medium of Mohan and Schaad (1987). Plates were incubated at room temperature (c. 20°C), marked for bacterial growth after three days, and re-checked after five days.

Plates were scored for:

1. bacterial growth consistent with the appearance of Psa
2. bacterial growth not consistent with the appearance of Psa
3. no bacterial growth.

2.2 qPCR

DNA extractions were done on isolations that potentially contained Psa. The 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.

2.3 Statistical analysis

Paired results were tabulated as 0 = no Psa and 1 = Psa positive. McNemar's Test with Yates' Continuity Correction was used on the data. This test is applied to 2 × 2 contingency tables with a dichotomous trait (yes/no data), with matched pairs of subjects, in this case egg-nest/no egg-nest pairs from single vines, to determine whether the row and column marginal frequencies are equal. The Yate's Correction is applied in cases where df = 1 and there is a relatively small sample size.

3 Results

Table 2 shows the number of canes in each block that were infected by Psa.

Table 2. Numbers of *Pseudomonas syringae* pv. *actinidiae*-positive canes, with and without cicada egg-nests, in each tested kiwifruit block.

		# of Psa-positive canes	
block	cultivar	egg-nest	no egg-nest
1	‘Hort16A’	7/25	6/25
2	‘Hort16A’	11/25	5/25
3	‘Hort16A’	2/25	0/25
4	‘Hayward’	5/25	0/25
5	‘Hayward’	1/25	0/25
6	‘Hayward’	0/25	0/25

Psa was significantly more likely to be isolated from canes with egg-nests than canes without egg-nests in one out of three ‘Hort16A’ blocks (*P*= 0.052) and one out of three ‘Hayward’ blocks (*P*= 0.044) (Table 3). When the three blocks of each cultivar were combined, Psa was significantly more likely to be isolated from canes with egg-nests than canes without egg-nests for both ‘Hort16A’ (*P*= 0.051) and for ‘Hayward’ (*P*= 0.025) (Table 4).

Table 3 Number of isolation attempts from 25 cane pairs (cicada egg-nest/no egg-nest) per orchard showing concordance or discordance between egg nest occurrence and *Pseudomonas syringae* pv. *actinidiae* isolation. *P*-values relate to McNemar’s Test with Yate’s Correction on the paired isolations.

Block	*Actinidia* cultivar			no egg-nest		McNemar Test with Yate's Correction	*P*-value
				no Psa	*Psa*		
1	‘Hort16A’	egg-nest	*no Psa*	14	4	0.028	0.868
			Psa	5	2		
2	‘Hort16A’	egg-nest	*no Psa*	13	1	3.781	0.052
			Psa	7	4		
3	‘Hort16A’	egg-nest	*no Psa*	23	0	1.125	0.289
			Psa	2	0		
4	‘Hayward’	egg-nest	*no Psa*	20	0	4.050	0.044
			Psa	5	0		
5	‘Hayward’	egg-nest	*no Psa*	24	0	0.250	0.617
			Psa	1	0		
6	‘Hayward’	egg-nest	*no Psa*	25	0	-	-
			Psa	0	0		

Table 4 Number of isolation attempts from 75 cane pairs (cicada egg-nest/no egg-nest) per cultivar (three 'Hort16A' blocks combined and three 'Hayward' blocks combined) showing concordance or discordance between egg nest occurrence and *Pseudomonas syringae* pv. *actinidiae* isolation. *P*-values relate to McNemar's Test with Yate's Correction on the paired isolations.

Block	*Actinidia* cultivar			no egg-nest		McNemar Test with Yate's Correction	*P*-value
				no Psa	*Psa*		
ALL	'Hort16A'	egg-nest	*no Psa*	50	5	3.803	0.051
			Psa	14	6		
ALL	'Hayward'	egg-nest	*no Psa*	69	0	5.042	0.025
			Psa	6	0		

4 Discussion

A previous artificial inoculation study showed that Psa can enter canes of the kiwifruit cultivars 'Hort16A' and 'Hayward' through cicada egg-nest wounds (Tyson et al. 2012). Although that work confirmed that cicada egg-nests are potential infection sites for Psa, the extent of the risk that they represent was not determined. In this study, natural Psa infection of cicada egg-nests was investigated by comparing the rate of infection of canes with cicada egg-nests with the rate of infection of the adjacent, unwounded canes.

Although there was a tendency for canes with egg-nests being more likely to be infected with Psa than canes without egg-nests, this was only significant within two kiwifruit blocks. There was, however, a significant difference for each cultivar between the infection rates of egg-nest canes and unwounded canes when all the vines in the three 'Hort16A' or all the vines in the three 'Hayward' blocks were combined.

This work suggests that some entry in the field is through cicada egg-nests. There was a higher rate of infection in the egg-nests than in the adjacent unwounded canes, so it is likely that the egg-nest wounds were the entry point for at least some of the infections, particularly in the 'Hayward' vines.

Infection rates appeared to be lower in the 'Hayward' blocks. This is a basic difference between this trial and the previous laboratory trial (Tyson et al. 2012), where no significant difference was found between Psa infection rates of cicada egg-nests on 'Hort16A' and 'Hayward' canes. It may be that lower infection rates in 'Hayward' canes are due to lower natural Psa inoculum in the 'Hayward' blocks.

Symptomless infection was found in unwounded 'Hort16A' canes, but not in unwounded 'Hayward' canes in this study. Although there was a low infection rate overall, this raises questions around whether 'Hayward' canes need wounding for Psa to enter. In addition, disease is known to develop more slowly in 'Hayward'; these were only one-year-old canes.

Psa DNA has been detected on cicadas (K. Everett, pers. comm.); however, it is not yet known whether cicadas can carry live Psa from vine to vine. The amount of Psa inoculum needed to initiate infection in relation to the amount cicadas may carry is unknown. It is likely that the egg-nest wound provides a site of entry for Psa, whether or not the bacteria are vectored by cicadas.

There was not a large difference in incidence of Psa infection between 'Hort16A' canes with egg-nests and those with no egg-nests. This suggests that where Psa inoculum is high in orchards with highly susceptible cultivars such as 'Hort16A', insect wounds may be of low importance in the epidemiology of this disease. However, for more tolerant cultivars such as 'Hayward', and potentially 'Yesy002' (commonly known as Gold3, now marketed as ZESPRI® SUNGOLD), cicada egg-nests may be important as infection sites.

Any wound is likely to be an entry point for Psa. Protection of wounds with copper and other ameliorants and the minimisation of wounding by control of cicadas are important considerations for disease management.

5 Acknowledgements

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Appendix – raw data

Block	Cultivar	Replicate	egg-nest	control
1	'Hort16A'	1	0	0
1	'Hort16A'	2	0	0
1	'Hort16A'	3	0	0
1	'Hort16A'	4	0	0
1	'Hort16A'	5	0	0
1	'Hort16A'	6	0	**1**
1	'Hort16A'	7	**1**	**1**
1	'Hort16A'	8	0	**1**
1	'Hort16A'	9	0	**1**
1	'Hort16A'	10	**1**	0
1	'Hort16A'	11	**1**	0
1	'Hort16A'	12	0	0
1	'Hort16A'	13	**1**	0
1	'Hort16A'	14	**1**	**1**
1	'Hort16A'	15	**1**	0
1	'Hort16A'	16	0	**1**
1	'Hort16A'	17	**1**	0
1	'Hort16A'	18	0	0
1	'Hort16A'	19	0	0
1	'Hort16A'	20	0	0
1	'Hort16A'	21	0	0
1	'Hort16A'	22	0	0
1	'Hort16A'	23	0	0
1	'Hort16A'	24	0	0
1	'Hort16A'	25	0	0
2	'Hort16A'	1	**1**	0
2	'Hort16A'	2	0	0
2	'Hort16A'	3	**1**	**1**
2	'Hort16A'	4	0	0
2	'Hort16A'	5	0	0
2	'Hort16A'	6	**1**	0
2	'Hort16A'	7	0	0
2	'Hort16A'	8	**1**	0
2	'Hort16A'	9	0	0
2	'Hort16A'	10	0	0
2	'Hort16A'	11	**1**	**1**
2	'Hort16A'	12	**1**	0
2	'Hort16A'	13	0	0
2	'Hort16A'	14	**1**	**1**
2	'Hort16A'	15	**1**	0
2	'Hort16A'	16	0	0

Block	Cultivar	Replicate	egg-nest	control
2	'Hort16A'	17	**1**	0
2	'Hort16A'	18	0	0
2	'Hort16A'	19	0	0
2	'Hort16A'	20	0	0
2	'Hort16A'	21	0	0
2	'Hort16A'	22	**1**	0
2	'Hort16A'	23	**1**	**1**
2	'Hort16A'	24	0	**1**
2	'Hort16A'	25	0	0
3	'Hort16A'	1	0	0
3	'Hort16A'	2	**1**	0
3	'Hort16A'	3	0	0
3	'Hort16A'	4	0	0
3	'Hort16A'	5	0	0
3	'Hort16A'	6	0	0
3	'Hort16A'	7	0	0
3	'Hort16A'	8	0	0
3	'Hort16A'	9	0	0
3	'Hort16A'	10	0	0
3	'Hort16A'	11	0	0
3	'Hort16A'	12	0	0
3	'Hort16A'	13	0	0
3	'Hort16A'	14	0	0
3	'Hort16A'	15	0	0
3	'Hort16A'	16	0	0
3	'Hort16A'	17	0	0
3	'Hort16A'	18	0	0
3	'Hort16A'	19	0	0
3	'Hort16A'	20	0	0
3	'Hort16A'	21	**1**	0
3	'Hort16A'	22	0	0
3	'Hort16A'	23	0	0
3	'Hort16A'	24	0	0
3	'Hort16A'	25	0	0
4	'Hayward'	1	0	0
4	'Hayward'	2	0	0
4	'Hayward'	3	**1**	0
4	'Hayward'	4	0	0
4	'Hayward'	5	**1**	0
4	'Hayward'	6	0	0
4	'Hayward'	7	0	0
4	'Hayward'	8	0	0
4	'Hayward'	9	0	0

Block	Cultivar	Replicate	egg-nest	control
4	'Hayward'	10	0	0
4	'Hayward'	11	0	0
4	'Hayward'	12	**1**	0
4	'Hayward'	13	0	0
4	'Hayward'	14	**1**	0
4	'Hayward'	15	0	0
4	'Hayward'	16	0	0
4	'Hayward'	17	0	0
4	'Hayward'	18	0	0
4	'Hayward'	19	**1**	0
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5	'Hayward'	15	0	0
5	'Hayward'	16	0	0
5	'Hayward'	17	**1**	0
5	'Hayward'	18	0	0
5	'Hayward'	19	0	0
5	'Hayward'	20	0	0
5	'Hayward'	21	0	0
5	'Hayward'	22	0	0
5	'Hayward'	23	0	0
5	'Hayward'	24	0	0
5	'Hayward'	25	0	0
6	'Hayward'	1	0	0
6	'Hayward'	2	0	0

Block	Cultivar	Replicate	egg-nest	control
6	'Hayward'	3	0	0
6	'Hayward'	4	0	0
6	'Hayward'	5	0	0
6	'Hayward'	6	0	0
6	'Hayward'	7	0	0
6	'Hayward'	8	0	0
6	'Hayward'	9	0	0
6	'Hayward'	10	0	0
6	'Hayward'	11	0	0
6	'Hayward'	12	0	0
6	'Hayward'	13	0	0
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6	'Hayward'	23	0	0
6	'Hayward'	24	0	0
6	'Hayward'	25	0	0