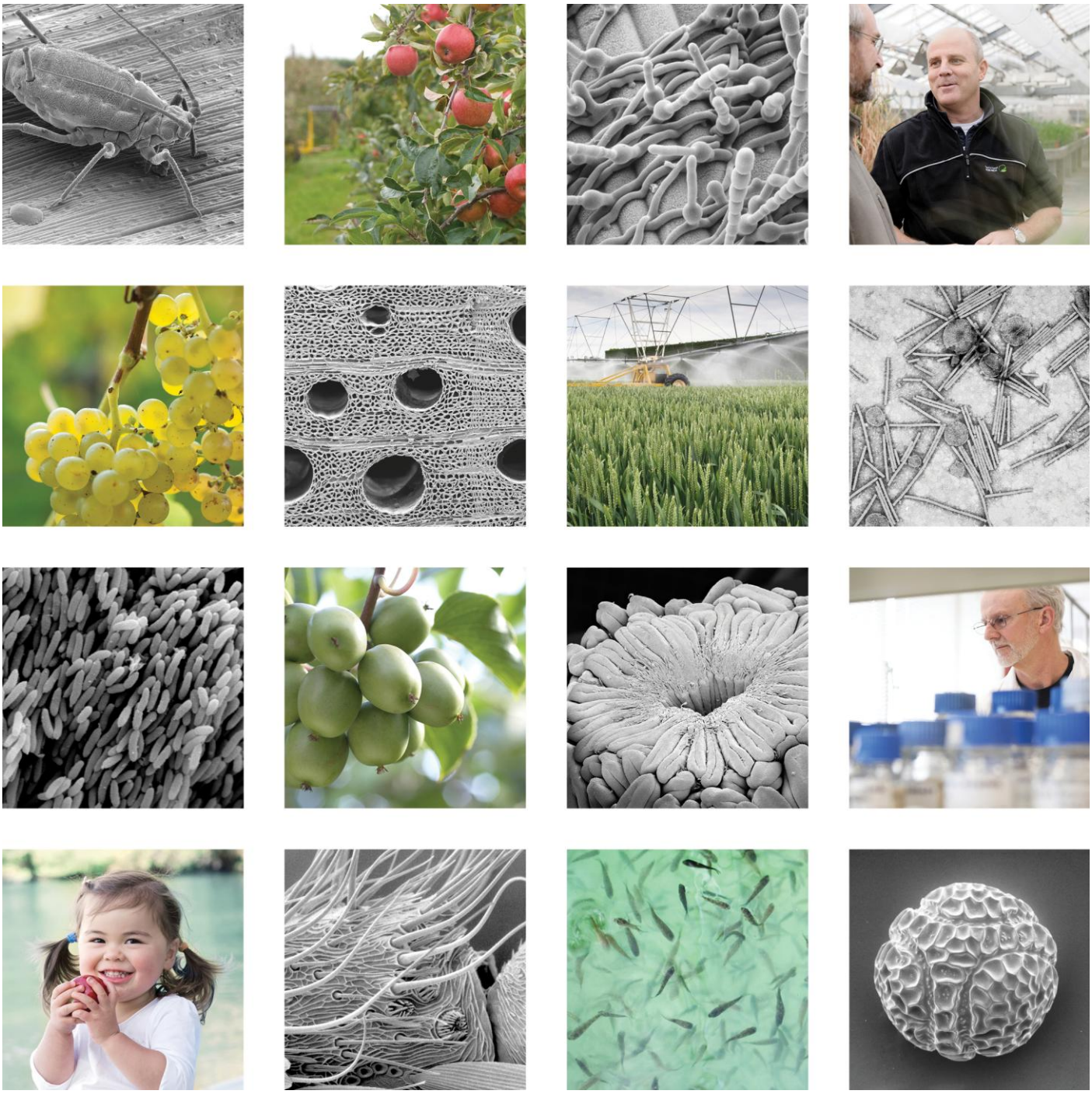
PFR SPTS No. 8457

Risk of plant debris as a *Pseudomonas syringae* pv. *actinidiae* inoculum source (ZESPRI-KRIP 2012 Objective 1)

Tyson JL, Curtis CL, Manning MA, Dobson SJ, McKenna CE, Beresford RM

May 2013



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Authors:
Tyson JL, Curtis CL, Manning MA, Beresford RM
Plant & Food Research, Mt Albert

Dobson SJ, McKenna CE
Plant & Food Research, Te Puke

PFR SPTS No. 8457

Report approved by:
Joy Tyson
Scientist, Pathology and Applied Mycology
Date: May 2013

Bob Fullerton
Science Group Leader, Pathology and Applied Mycology
Date: May 2013

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Executive summary

Risk of plant debris as a *Pseudomonas syringae* pv. *actinidiae* inoculum source (ZESPRI-KRIP 2012 Objective 1)

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Previous work has shown that *Pseudomonas syringae* pv. *actinidiae* (Psa) survives in kiwifruit plant debris on the orchard floor at least until spring, but it is not known if this material poses a risk for re-infection of kiwifruit vines. This study used trap plates and trap plants to determine the importance of fallen leaves and winter prunings on the orchard floor as inoculum sources.

Psa was identified from trap plates exposed during the first four weeks of the trial (30 July - 20 August 2012), but only once thereafter. Psa-positive trap plates were only found when the plates were exposed during rain events; however, they were not found during every rain-period. A Psa-positive result was never returned from the highest trap plates located 100 cm above the debris, and only intermittently from the lower plates, which were 15 cm and 50 cm above the debris.

Leaf lesions developed only on the trap plants exposed during the first week of the 20-week trial (week beginning 30 July 2012).

In this trial, Psa was found to be splashed upwards from the debris during rain, although not in great amounts and it was never splashed higher than the second trap plate (50 cm above the debris).

In this trial, plant debris appears to have represented a low risk in terms of an inoculum source and would not have posed a major risk for re-infection of any surrounding kiwifruit vines at the time of budburst (September).

This study constituted Objective 1 of a larger study currently being run with KRIP funding (2012-2013). The larger project complements this study, with aerosol traps and kiwifruit trap plants being placed in infected blocks, beneath and above the canopy. This allows continuous monitoring of inoculum production and infection periods. Weather data are continuously collected from the weather station at the PFR Te Puke Research Orchard (TPRO) to compare with inoculum production and infection periods. This will give a more complete understanding of the basic requirements of Psa for inoculum production and infection, increasing our understanding of the life cycle of Psa, potentially helping to refine assays and allowing for improvements to the KVH Psa-V Risk Model.

For further information please contact:

Joy Tyson
The New Zealand Institute for Plant & Food Research Ltd
Plant & Food Research Mt Albert
Private Bag 92169, Auckland 1142
NEW ZEALAND
Tel: +64-9-925-7139
Fax: +64-9-925-7001
Email: Joy.Tyson@plantandfood.co.nz

1 Introduction

The bacterial plant pathogen *Pseudomonas syringae* pv. *actinidiae* (Psa) causes a devastating disease of *Actinidia* spp. in a number of countries around the world. Psa was first found in New Zealand in November 2010, in a kiwifruit orchard in Te Puke (Everett et al. 2011). In subsequent years, further infected orchards and regions have been identified across the country. Psa is now a serious threat to the New Zealand kiwifruit industry and a significant amount of research is underway to address the problem. One of these areas of research is investigating the sources of inoculum within kiwifruit orchards.

Other pathovars of *Pseudomonas syringae* have been found to survive in plant debris. For example, *Pseudomonas syringae* pv. *lachrymans*, the cause of angular leaf spot of cucumber, can survive in plant debris for at least eight months after harvest (Bhat 2009), while Hollaway and Bretag (1997) confirmed that *Pseudomonas syringae* pv. *pisi* can survive on field pea trash for two seasons (78 weeks) and that infected pea trash is a potential source of inoculum for a following pea crop.

Bacterial canker of cherry trees can be incited by two different pathogens: *Pseudomonas syringae* and *Pseudomonas morsprunorum* (*P. syringae* pv. *morsprunorum*). Latorre and Jones (1979) found that *Pseudomonas syringae* was able to be isolated from plant refuse collected from under cherry trees, whereas *P. morsprunorum* was not. They concluded that plant refuse was a possible source for primary inoculum of bacterial canker caused by *P. syringae*, but not by *P. morsprunorum*.

It has been previously found that Psa survives in kiwifruit plant debris on the orchard floor at least until spring (Tyson et al. 2011, 2012), but it is not known if this material poses a risk for re-infection. There is increased interest by growers in the best way to manage the huge amount of plant material, such as fallen leaves and prunings, during the changeover to less susceptible cultivars in orchards.

This study aimed to determine the importance of fallen leaves and winter prunings on the orchard floor as an inoculum source.

2 Methods

Four frames containing fallen kiwifruit leaves and pruning debris with symptoms of Psa infection were set up next to the weather station at Te Puke Research Orchard (TPRO) on Friday 27 July 2012. This area was set well away from kiwifruit canopies. This allowed the risk of debris as an inoculum source to be assessed in isolation from the risk of inoculum from infected vines.

Each frame was 1.2 m x 1.2 m in size and filled with a mixture of kiwifruit leaves with Psa leaf spot symptoms and cane/leader wood with dieback or orange ooze/cankers. The trial plan is shown in Figure 1 and the actual setup is shown in Figure 2.

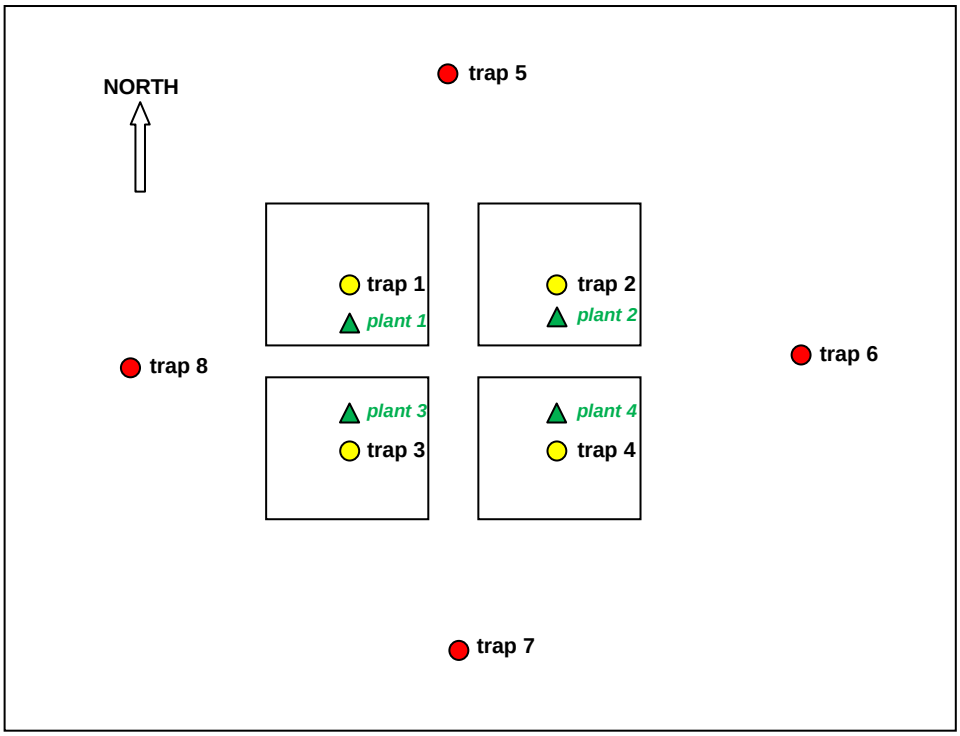

Figure 1. Plan of debris trial, showing debris frames, positions of aerosol traps and trap plants, Te Puke Research Orchard 2012.

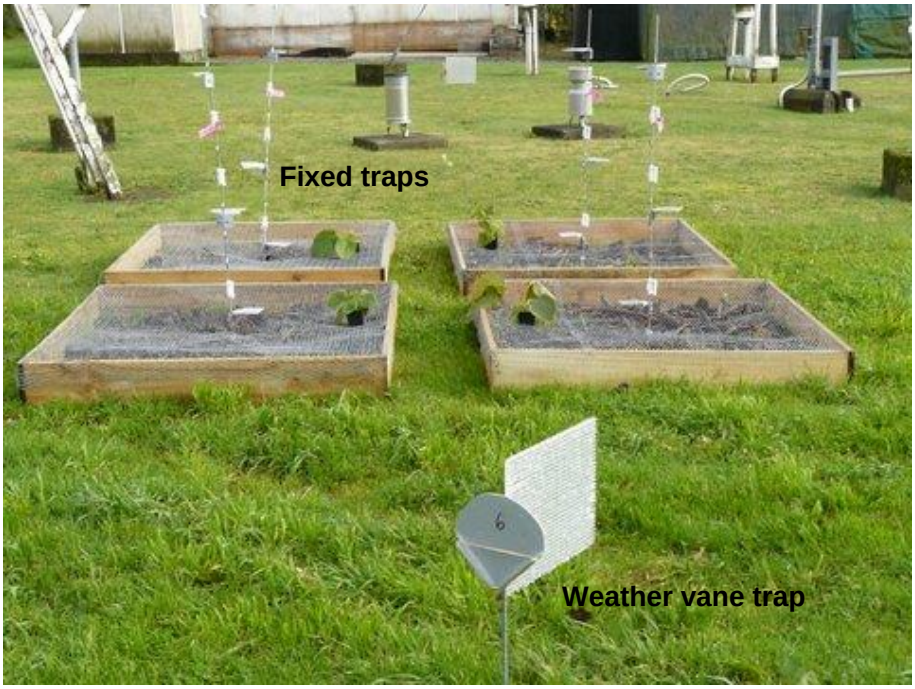

Figure 2. Debris trial setup, Te Puke Research Orchard 2012.

2.1 Trap plates

Methods have been previously developed to capture and identify Psa from the air using passive aerosol traps (Tyson et al. 2013). Two types of trap were used:

1) A swinging vane aerosol trap, with a plate of bacteriological growth media attached to a weather vane to keep it facing into the wind on the top of a metal stake to detect windborne inoculum (Figure 3), and

2) A metal stake with fixed positions for agar plates at three heights (15 cm, 50 cm, 100 cm above the debris), all pointing downwards, to detect upwards 'splash-dispersal' of the bacteria.

Figure 3. Passive swinging aerosol trap under a kiwifruit canopy (winter 2012).

One set of fixed downward facing traps was placed in each debris frame. In addition, weathervane-type traps were set up around the outside of the frames (3 m distance) in four positions (N, S, E and W). These operated at the same time in order to indicate direction of inoculum spread. This gave a total of 16 trap plates - 12 in the stationary traps and four in the weathervane-style traps around the edges of the debris frames.

Plates were put out four days in each week (Monday-Thursday) and removed after 24 hours (0900 h - 0900 h), for 20 weeks over winter and spring 2012. After removal from the traps, the plates were incubated at 20°C for three days. Colonies with morphology indicative of Psa were counted and DNA extractions were made on the mixed-colony plates. These were then tested for Psa by quantitative Polymerase Chain Reaction (qPCR), using the primers PsaF3/R4 of Rees-George et al. (2010).

In this study, a Cp (crossing point or threshold value) value below 30 was interpreted as a Psa-positive result and a Cp value above 35 as a negative/not-detected result. Cp values between 30 and 35 are recorded as 'weak positive', which indicate either very low quantities of Psa or an uncertain result.

2.2 Trap plants

A single Psa-free *Actinidia chinensis* 'Hort16A' plant, raised from tissue culture, was placed in each frame each week (a total of four plants). The plants were placed in the frames each Monday morning and were collected on Friday morning. After exposure in the field, the plants were incubated in the TPRO PC2-2 lab for 21 days and then assessed for symptom expression (leaf spots). Any leaves that developed leaf spots typical of Psa were sent to Mt Albert Research Centre (MARC) for further diagnosis.

Symptomatic areas were excised, macerated in 200 µL bacteriological saline (BS) and left for five minutes, after which 100 µL of the resulting suspension was spread across a semi-selective agar medium. The isolation plates were incubated at 20°C for 72 hours, and then assessed for bacterial growth. DNA extractions were made on the mixed-colony plates and qPCR identification carried out.

2.3 Debris samples (leaves only)

Isolations and qPCR identification were carried out weekly on the plant debris below the traps (the inoculum source). Single leaves were taken from each frame at each sampling date. Cankered canes and woody tissue were not tested as this would have resulted in a dwindling 'inoculum source' over the course of the trial (20 weeks).

2.4 Weather data

Weather data, including rainfall and wind direction, were collected from the immediately adjacent weather station at TPRO over the period of trapping, and compared with trap data. The weather data were downloaded from the National Institute of Water and Atmospheric Research Ltd (NIWA) National Climate Database (CliFlo[1]) for the period of the trial (Te Puke Ews, station no. 12428, latitude 37.822, longitude 176.324). Plates and trap plants were exposed in the field from 30 July 2012 until 14 December 2012 (20 weeks).

[1] CliFlo http://cliflo.niwa.co.nz/

3 Results and discussion

This trial aimed to determine the importance of kiwifruit plant debris as a Psa inoculum source. The relationship between splash dispersal (distance from inoculum source), time (degradation of the litter) and weather (environmental factors) was studied.

A summary of the qPCR results from leaf litter, trap plates and trap plants is given in Table 1.

Table 1. Summary of qPCR results for *Pseudomonas syringae* pv. *actinidiae* (Psa) from *Actinidia* leaf litter, trap plates and 'Hort16A' trap plants over the period 30 July – 10 December 2012.

4-day period starting:	Leaf litter positive (weak positive)	Fixed trap plate positive (weak positive)	Weathervane plate positive (weak positive)	Trap plant positive	total rain (mm) over 4-day period
Monday, 30 July 2012	0 (0)	0 (2)	1 (0)	2	105.6
Monday, 6 August 2012	1 (0)	1 (3)	0 (1)	0	14.2
Monday, 13 August 2012	0 (0)	2 (3)	1 (4)	0	10.0
Monday, 20 August 2012	0 (1)	2 (0)	0 (0)	0	16.4
Monday, 27 August 2012	0 (1)	0 (0)	0 (0)	0	1.0
Monday, 3 September 2012	0 (0)	0 (0)	0 (0)	0	25.4
Monday, 10 September 2012	0 (2)	0 (0)	0 (0)	0	0.8
Monday, 17 September 2012	0 (0)	0 (0)	0 (0)	0	0.0
Monday, 24 September 2012	1 (0)	0 (0)	0 (0)	0	1.4
Monday, 1 October 2012	0 (0)	0 (0)	0 (0)	0	5.6
Monday, 8 October 2012	0 (1)	1 (0)	0 (0)	0	13.6
Monday, 15 October 2012	0 (0)	0 (0)	0 (0)	0	16.4
Tuesday, 23 October 2012	0 (0)	0 (0)	0 (0)	0	0.4
Monday, 29 October 2012	0 (0)	0 (0)	0 (0)	0	0.0
Monday, 5 November 2012	0 (0)	0 (0)	0 (0)	0	0.0
Monday, 12 November 2012	0 (0)	0 (0)	0 (0)	0	8.4
Monday, 19 November 2012	0 (1)	0 (0)	0 (0)	0	0.0
Monday, 26 November 2012	0 (0)	0 (0)	0 (0)	0	0.0
Monday, 3 December 2012	0 (0)	0 (0)	0 (0)	0	21.8
Monday, 10 December 2012	1 (1)	0 (1)	0 (1)	0	15.6

Figure 4 shows daily weather data from the Te Puke Research Orchard weather station: rainfall and maximum and minimum temperatures.

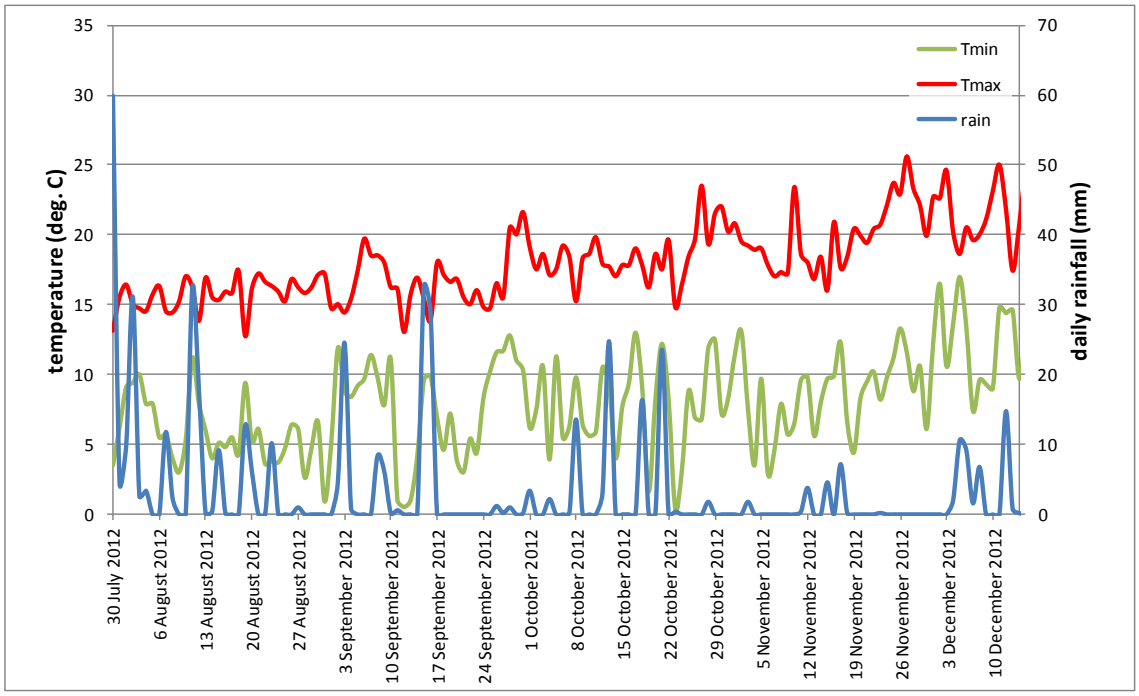

Figure 4. Weather data from the Te Puke Research Orchard weather station: daily rainfall and daily maximum and minimum temperatures.

3.1 Trap plates

Psa was identified from the trap plates consistently during the first four weeks of the trial (30 July – 20 August 2012), but only once thereafter. Psa-positive trap plates were only found when the plates were exposed during rain, but they were not found during every rain-period.

On the fixed plate traps, a Psa-positive result was never returned from the highest plates (100 cm above the debris) and only intermittently from the lower plates (15 cm and 50 cm above the debris). Almost all the inoculum activity on the trap plates, including weak-positive results, was detected in the first four weeks of the trial.

In this trial, Psa was found to be splashed upwards from the debris during rain, although not in great amounts and it was never splashed higher than the second trap plate (50 cm above the debris).

3.2 Trap plants

Leaf lesions developed on the 'Hort16A' trap plants only during the first week of the 20-week trial (week beginning 30 July 2012).

3.3 Debris samples (leaves only)

The survival of Psa in fallen leaves and cane prunings was previously investigated during winter 2011 (Tyson et al. 2011, 2012). In that trial, all leaves yielded live Psa at leaf-fall. Although detection frequency declined over time, especially after 5-6 weeks, Psa was still isolated from leaf litter 15 weeks (9 September 2011) after leaf-fall, and from cane prunings 11 weeks after winter pruning (3 October 2011). The results

from the 2011 trial indicated that the pathogen overwinters readily in leaf litter and pruning debris, representing a potential inoculum source for infection of new spring growth.

In this trial, only the debris leaves were assayed for live Psa over time. There were very few Psa-positive results. A comparison of the 2011 and 2012 leaf debris isolation results is shown in Figure 5. The 2012 trial was started ten weeks later in the season than the 2011 trial and the first assays of the 2012 trial corresponded with the time at which there had been a significant decline in recovery of Psa in the 2011 trial. Psa appeared to be at, or below the level of detection by that time.

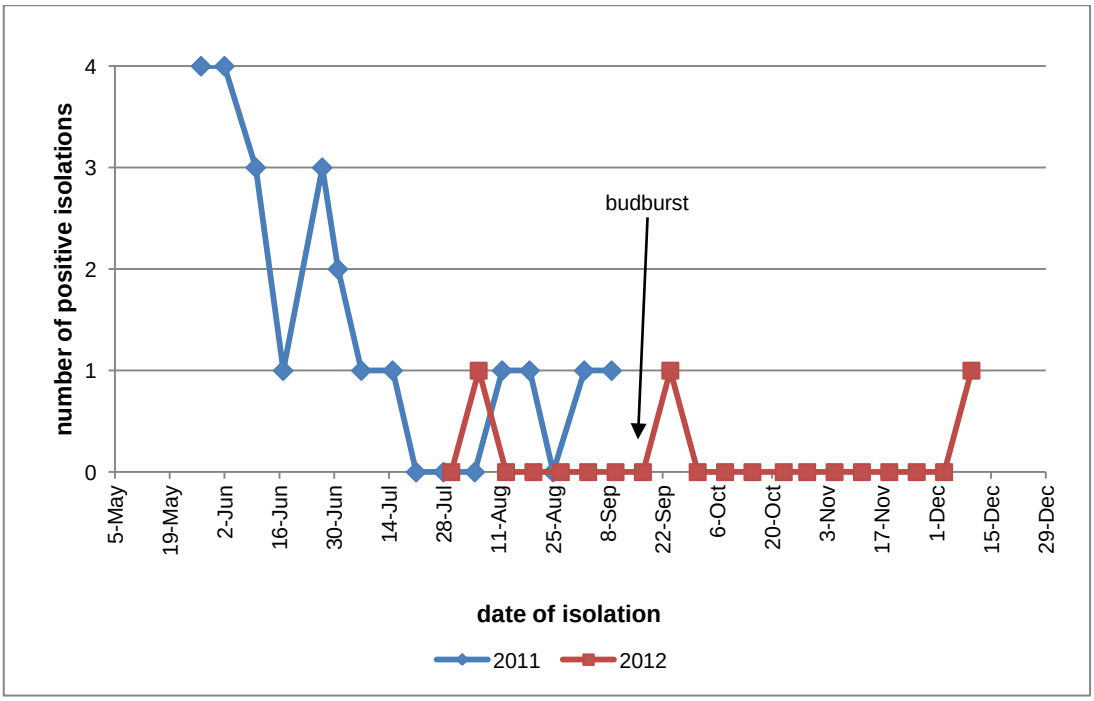

Figure 5. Frequency of isolation of viable *Pseudomonas syringae* pv. *actinidiae* (Psa) from four separate samples of kiwifruit leaf litter held in frames in the orchard over the periods 25 May–1 September 2011 and 30 July-14 December 2012.

4 Conclusions

From the results of this trial, it is concluded that plant debris is unlikely to pose a major risk of re-infection of surrounding kiwifruit vines at the time of budburst (September). Although the cankered and dieback pruning material in the frames is likely to have remained active for longer than the leaves, if this material followed the pattern seen in 2011, Psa was never recovered from 100 cm above the debris, and the trap plants developed lesions only in the first week of the trial. The debris in this trial appears to represent a low risk in terms of an inoculum source.

This study constitutes Objective 1 of a larger study currently being run with KRIP funding (2012-2013). The larger project complements this study, with aerosol traps and kiwifruit trap plants being placed in infected blocks, beneath and above the canopy. This allows continuous monitoring of inoculum production and infection periods.

Weather data are continuously collected from the weather station at TPRO to compare with inoculum production and infection periods. This will give a more complete understanding of Psa inoculum production and infection, increasing our understanding of the life cycle of Psa, and helping to refine assays and allowing for improvements to the KVH Psa-V Risk Model.

5 References

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Appendix 1 – Te Puke Research Orchard weather data

NIWA National Climate Database: station no. 12428, latitude 37.822, longitude 176.324. Daily weather data from 30 July 2012 until 14 December 2012 (20 weeks).

Date	Time (NZST)	Tmax (°C)	Tmin (°C)	Tgmin (°C)	Rain (mm)
20-Jul-12	900	15.2	3	-1.5	0
21-Jul-12	900	15.5	7	7.2	0
22-Jul-12	900	13.8	10	9.8	6
23-Jul-12	900	14.5	10	10	74.4
24-Jul-12	900	14	11.4	11.1	84.6
25-Jul-12	900	15.7	10.6	7.2	8.4
26-Jul-12	900	15.7	8.1	5.6	0
27-Jul-12	900	15.1	1.8	-2.6	0
28-Jul-12	900	14.2	3.1	-1.1	0
29-Jul-12	900	14	6.5	2.5	5
30-Jul-12	900	13.8	10.8	10.9	19.4
31-Jul-12	900	13.1	3.5	0.2	60
1-Aug-12	900	15.5	6.2	2.9	4.6
2-Aug-12	900	16.4	9.1	5.5	9.8
3-Aug-12	900	15	9.4	8.5	31.2
4-Aug-12	900	14.7	10	7.9	2.6
5-Aug-12	900	14.5	7.9	5.6	3.4
6-Aug-12	900	15.7	7.9	4.7	0
7-Aug-12	900	16.3	5.5	1.8	0
8-Aug-12	900	14.5	5.7	2.3	11.8
9-Aug-12	900	14.4	4	0.7	2.4
10-Aug-12	900	15.2	3	-1.2	0
11-Aug-12	900	17	5.3	1.1	0
12-Aug-12	900	16.2	11.2	11.3	32.4
13-Aug-12	900	13.8	7.9	4.9	17.8
14-Aug-12	900	16.9	6	2.5	0.2
15-Aug-12	900	15.5	4	0.9	0.4
16-Aug-12	900	15.3	5.1	1.6	9.2
17-Aug-12	900	15.9	4.8	0.8	0.2
18-Aug-12	900	15.8	5.5	1.6	0
19-Aug-12	900	17.4	4.3	0.6	0
20-Aug-12	900	12.7	9.4	9.6	12.8
21-Aug-12	900	16.1	5.2	1.4	6.2
22-Aug-12	900	17.2	6.1	1.9	0
23-Aug-12	900	16.6	3.6	-0.6	0
24-Aug-12	900	16.3	3.8	-0.7	10.2
25-Aug-12	900	15.9	3.7	-0.6	0
26-Aug-12	900	15.2	4.7	0.8	0
27-Aug-12	900	16.8	6.4	3.3	0

Date	Time (NZST)	Tmax (°C)	Tmin (°C)	Tgmin (°C)	Rain (mm)
28-Aug-12	900	16.2	6.1	2.2	1
29-Aug-12	900	15.8	2.6	-1.7	0
30-Aug-12	900	16.2	4.7	0.6	0
31-Aug-12	900	17.1	6.6	1.9	0
1-Sep-12	900	17.2	0.9	-3.3	0
2-Sep-12	900	14.7	5.1	0.6	0
3-Sep-12	900	15	11.9	10.1	4.6
4-Sep-12	900	14.4	8.8	7	24.6
5-Sep-12	900	15.4	8.4	6.4	0.8
6-Sep-12	900	17.4	9.2	5.2	0
7-Sep-12	900	19.7	9.7	6.2	0
8-Sep-12	900	18.5	11.4	8.6	0
9-Sep-12	900	18.5	9.8	7.9	8.4
10-Sep-12	900	18	7.8	5.1	6.2
11-Sep-12	900	16.2	11.1	8.8	0.2
12-Sep-12	900	16.1	1	-2.3	0.6
13-Sep-12	900	13	0.5	-3.8	0
14-Sep-12	900	15.7	1	-2.8	0
15-Sep-12	900	16.9	4.1	0	0
16-Sep-12	900	15.4	9.6	9.7	32.6
17-Sep-12	900	13.8	9.9	6.6	29
18-Sep-12	900	18	6.9	2.9	0
19-Sep-12	900	17.1	4.6	1.1	0
20-Sep-12	900	16.6	7.2	3.3	0
21-Sep-12	900	16.8	3.8	-0.6	0
22-Sep-12	900	15.5	3	-1.2	0
23-Sep-12	900	15	5.4	0.7	0
24-Sep-12	900	16	4.4	0.5	0
25-Sep-12	900	14.8	8.3	6.4	0
26-Sep-12	900	14.7	10.2	9	0
27-Sep-12	900	16.5	11.6	10.9	1.2
28-Sep-12	900	15.5	11.7	10.2	0.2
29-Sep-12	900	20.5	12.8	12.6	1
30-Sep-12	800	20	11	8.1	0
1-Oct-12	800	21.6	10.3	7.4	0.2
2-Oct-12	800	19.1	6.2	2.8	3.4
3-Oct-12	800	17.5	7.5	3.8	0
4-Oct-12	800	18.6	10.6	8.7	0
5-Oct-12	800	17.1	3.9	0.1	2.2
6-Oct-12	800	17.5	11.3	10.4	0
7-Oct-12	800	19.2	5.5	2.8	0
8-Oct-12	800	18.4	6.3	2.6	0.2
9-Oct-12	800	15.2	9.8	10.1	13.6

Date	Time (NZST)	Tmax (°C)	Tmin (°C)	Tgmin (°C)	Rain (mm)
10-Oct-12	800	18.3	6.4	3	0
11-Oct-12	800	18.6	5.6	1.9	0
12-Oct-12	800	19.8	5.9	1	0
13-Oct-12	800	17.9	10.5	7.5	3
14-Oct-12	800	17.7	9.2	6.7	24.8
15-Oct-12	800	17	4	0.8	0
16-Oct-12	800	17.8	7.7	4.4	0
17-Oct-12	800	17.8	9.4	6.2	0
18-Oct-12	800	19	13	11.3	0
19-Oct-12	800	17.8	9.1	6.6	16.4
20-Oct-12	800	16.2	1.6	-2.4	0
21-Oct-12	800	18.6	8.2	5.1	0
22-Oct-12	800	17.5	12.2	12.1	23.6
23-Oct-12	800	19.6	8.2	5.2	0
24-Oct-12	800	14.8	0.4	-3.5	0.4
25-Oct-12	800	16.4	3.1	-0.1	0
26-Oct-12	800	18.4	8.8	4.9	0
27-Oct-12	800	19.7	6.9	2.9	0
28-Oct-12	800	23.5	6.8	3.3	0
29-Oct-12	800	19.3	11.9	8.6	1.8
30-Oct-12	800	21.5	12.5	10.3	0
31-Oct-12	800	22	7.2	3.5	0
1-Nov-12	800	20.2	8.3	4.9	0
2-Nov-12	800	20.8	11.4	7.7	0
3-Nov-12	800	19.5	13.1	11.6	0
4-Nov-12	800	19.2	7.5	5.2	1.8
5-Nov-12	800	18.9	3.5	-0.8	0
6-Nov-12	800	19	9.7	6.1	0
7-Nov-12	800	17.8	2.9	-0.8	0
8-Nov-12	800	17	4.6	-0.1	0
9-Nov-12	800	17.3	7.9	5.1	0
10-Nov-12	800	17.2	5.7	1.6	0
11-Nov-12	800	23.4	6.5	2.7	0
12-Nov-12	800	18.6	9.6	7.2	0.4
13-Nov-12	800	18	9.8	9.3	3.8
14-Nov-12	800	16.8	5.6	2.1	0
15-Nov-12	800	18.4	8	5.1	0
16-Nov-12	800	16	9.7	6.5	4.6
17-Nov-12	800	20.9	9.9	7.6	0
18-Nov-12	800	17.6	12.3	9.8	7.2
19-Nov-12	800	18.4	6.6	2.4	0.2
20-Nov-12	800	20.4	4.4	0.3	0
21-Nov-12	800	19.9	8.3	3.4	0

Date	Time (NZST)	Tmax (°C)	Tmin (°C)	Tgmin (°C)	Rain (mm)
22-Nov-12	800	19.4	9.5	5.6	0
23-Nov-12	800	20.4	10.2	6.5	0
24-Nov-12	800	20.7	8.2	4	0.2
25-Nov-12	800	22.1	9.8	5.9	0
26-Nov-12	800	23.7	11.2	7.9	0
27-Nov-12	800	22.9	13.3	9.9	0
28-Nov-12	800	25.6	11.6	8	0
29-Nov-12	800	23.3	8.8	5	0
30-Nov-12	800	22.1	10.6	7.1	0
1-Dec-12	8:00	19.9	6.1	1.4	0
2-Dec-12	8:00	22.7	12.3	10.2	0
3-Dec-12	8:00	22.6	16.5	16.2	0
4-Dec-12	8:00	24.6	10.6	5.8	0
5-Dec-12	8:00	20.2	13.6	13.6	2
6-Dec-12	8:00	18.6	17	16.7	10.6
7-Dec-12	8:00	20.5	13.4	11.1	9.2
8-Dec-12	8:00	19.6	7.4	4.3	1.6
9-Dec-12	8:00	20	9.6	6.6	6.8
10-Dec-12	8:00	21.1	9.3	6.2	0
11-Dec-12	8:00	23.1	9	5.1	0
12-Dec-12	8:00	25	14.8	12.4	0
13-Dec-12	8:00	21.8	14.4	14.4	14.8
14-Dec-12	8:00	17.4	14.6	14.8	0.8

DISCOVER. INNOVATE. GROW.