



Psa epidemiology: Predictive modelling – Trap plant field
validation of Psa risk model

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PROJECT DETAILS

Project Title	Psa epidemiology: Predictive modelling – Trap plant field validation of Psa risk model
Project Protocol No./ Objective No.	
Project Leader	Alistair McKay
Research Requested / Contracted by	ZESPRI
Date (Month, Year)	December 2011

KEY QUESTION AND AIM

Plant & Food Research has developed a preliminary predictive model (Beresford 2011) to identify weather conditions conducive to infection of kiwifruit plants with, *Pseudomonas syringae* pv. *actinidiae* (Psa), that causes bacterial canker. The PSA risk model uses hourly wetness (or relative humidity), temperature and rainfall to calculate a daily risk index, *R*, which aims to identify days with weather conditions suitable for multiplication and infection of the Psa bacterium. The model is intended for use by NIWA to implement a web-based information system to deliver the model to the kiwifruit industry.

Computer models that use weather data to predict risk of infection by bacterial plant pathogens are used in several other crops for managing bacterial diseases. For example, *Cougarblight* (Smith, 1999) and *MARYBLIGHT* (Lightner and Steiner, 1993) are two versions of predictive models that have been developed to help manage fire blight of apple caused by *Erwinia amylovora*. For Psa of kiwifruit, the weather conditions that lead to infection and disease development have not been established, although wetness periods at or above a particular temperature are thought to be required. For bacterial canker of cherry, for example, a wetness period of 6 hr at temperatures greater than 15°C resulted in higher than 90% disease incidence in two year old Sweet Cherry twigs (*Prunus avium*) inoculated with *Pseudomonas syringae* pv. *syringae* Van Hall (Latorre et al 2002). Tomato plants (*Solanum lycopersicum*) inoculated over a range of temperatures from 10°C to 30°C with *Pseudomonas syringae* pv. *tomato*, developed up to 16.8% infection, after a 1 hr exposure at 100% relative humidity (RH) with infection increasing linearly to 45.6% infection after 24 hours (Gullino et al. 2009). Determination of the temperature and wetness requirements for infection of kiwifruit by Psa is required to test the preliminary Psa risk model and, if necessary, to modify the model to improve its accuracy.

This project evaluated the ability of the risk model to predict infection in kiwifruit orchards by using an exposed trap plant method. This method allowed the amount of infection occurring on exposed potted kiwifruit plants in the field during discrete periods of time (e.g. one week) to be related field weather conditions during that period. Following exposure, the plants were incubated in a laboratory where conditions were not suitable for further infection and the amount of leaf spotting that arose gave an indication of the suitability of the field conditions during exposure for infection. The trap plants were exposed under 'Hort16A' kiwifruit vines in Block 18 at Te Puke Research Orchard during early spring 2011. This block had been used for a copper efficacy trial during the preceding leaf fall and winter period and trap plants were exposed both under vines that had received copper and under vines that had not received copper.

METHODOLOGY

Trap plant material

'Gold' kiwifruit seedlings (*Actinidia chinensis* c.v. 'Hort16A') were propagated in 20 cm pots from seed at Plant and Food Research Kerikeri from June through July, 2011. At weekly intervals, from the 30 August to the 26 October, consignments of approximately 100 plants plants were transported to Te Puke Research Orchard (TPRO) for exposure within the Psa-infected kiwifruit orchard Block 18. The first trap plants were exposed in Block 18 on 1 September 2011 and weekly exposures continued for nine weeks, with the last plants removed on 3 November 2011.

Block 18 management

Block 18 at TPRO contained 340 pergola-trained kiwifruit vines with "strip-males", comprising 177 female 'Hort 16A' and 163 male vines of either CK2, or CK3. The block was planted in 2001 and had been managed in accordance with Zespri export protocols. Suspected PSA infected vines were first detected about the time of leaf fall in 2011 and 13 female vines had been removed prior to 1 June 2011. The date that canker or dieback symptoms of Psa (secondary symptoms) first appeared in each vine was recorded and each diseased vine was removed within 2 weeks of bacterial canker symptom appearance (Figure 1). At 23 November 2011, there were only 44 vines, comprising 30 females and 14 males remaining.

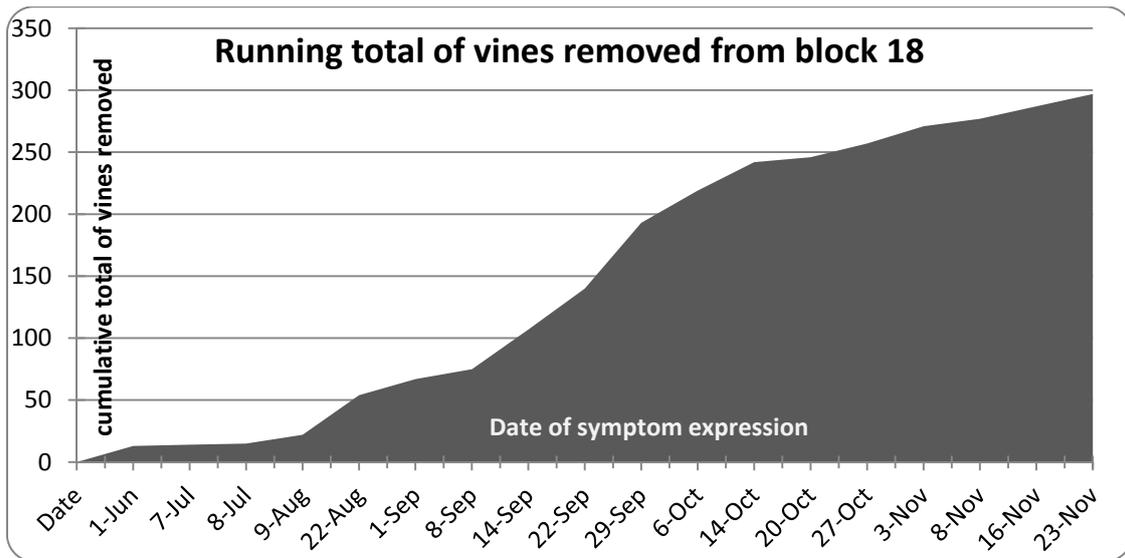


Figure 1. Cumulative total of vines removed from Block 18 at the Te Puke Research orchard because of PSA bacterial canker symptoms. Vines were removed as soon as was practical following the appearance of secondary symptoms. Prior to vine removal block 18 contained 340 vines.

Block 18 leaf-fall and winter copper treatments

Block 18 had been used for an investigation into the efficacy of copper as a bactericide against Psa during the preceding leaf fall and dormancy period. The copper product Kocide® Opti (300g/kg copper hydroxide) was applied at the rates of 0, 20, 40, 80 and 120 g/100L using a conventional commercial air-blast sprayer and the equivalent of 2000L/ha of water. These application rates represented 0.0x, 0.25x, 0.5x, 1.0x and 1.5x the recommended label rates for Kocide® Opti. Each treatment (application rate) was applied six times. Four applications were made at weekly intervals between 20% leaf fall on 15 June until 4 weeks before HiCane® application in late July. Two further applications were made on 13 August and 25 August, the final one being at the beginning of bud break. A randomized complete block design was used for the five copper treatments, each replicated eight times. Each experimental plot consisted of 3-4 vines. Treatments were applied only to rows containing female vines and the adjacent the male rows provided spatial buffers between the treated rows.

Weekly trap plant exposures

Trap plants were exposed at weekly intervals at eight locations beneath the vines in Block 18. At each location, six plants were placed into 30 cm x 60 cm aluminium baking trays with a modified wire scaffold to keep the plants upright. The plants were regularly watered by adding 2-4 cm of water to the bottom of each tray. Half of the plants (four replicates) were exposed beneath vines that had been treated with the high dose of Kocide® Opti (1.5 x label rate) and the other half beneath the non-copper treated control vines. Because of the removal of so many Psa-symptomatic vines from block 18 the trap plants were no longer exposed according to leaf-fall copper application

regime from week 7 through week 9, the plants were exposed beneath symptomatic vines, although not according to leaf fall copper application.

To determine if the source of inoculum was immediately proximate to infected kiwifruit vines or possibly from an adjacent orchard or other distant source, an additional four sets of trap plants were exposed at the TPRO weather station. By convention weather stations are positioned in an open grass covered area, so there were no infected kiwifruit vines within 50 m of the exposed plants. For each weekly exposure period an additional set of 24 control plants were placed in a location that was not directly exposed to PSA inoculum. For the first six weeks these controls were maintained in a PC2 laboratory on-site at the TPRO during each exposure period. From weeks 6 – 9 the control plants were grown on in the TPRO nursery under shade cloth.

Trap plant incubation after weekly exposure and symptom recording

Following the 7-day exposure periods the trap plants were returned to PC2-1 that is a certified PC2 laboratory facility at Te Puke Research Orchard for incubation and symptom expression. The plants were labelled and monitored daily and the number of days until the first symptoms appeared was recorded. Final symptom severity was recorded after 20 days using a visual estimation of the number of leaf spots on every infected leaf using categories of 0, 1-5, 6-20, 20-50, or >50 spots per leaf. The position of each leaf was also recorded.

Calculation of *R* index values.

The Psa infection risk model output of daily *R* index values were calculated during the trap plant exposure period from 1/9/2011 to 3/11/2011. The *R* (wetness >50%) and *R'* (relative humidity >94%) index values were calculated based on the multiplication rate index of Psa (*M*) derived using equation 1 (Appendix A1.1) over the current day and the two preceding days (potentially 72 hours) in which surface wetness was recorded. Surface wetness periods were defined by hours >94% relative humidity (RH) or hours >50% surface wetness recorded using the Te Puke Research Orchard's weather station wetness sensor. The model calculates the risk index (*R* or *R'*) for days with rainfall greater than 1.0 mm. The risk index value is the sum of hourly *M* values calculated for the two days leading up to and including the day with recorded rainfall.

KEY RESULTS

Bacterial leaf spot symptoms first appeared on the trap plants after 8-13 days of incubation in the PC2 laboratory at room temperature (Table 1). Using the PSA infection risk model, weekly totals of infection risk (*R'* or *R* index) were calculated for each week that plants were exposed based on the measured weather conditions. Leaf spot symptoms developed on trap plants for seven of the nine weeks that the plants were exposed (Table 1.). For all exposures pooled across copper treatments, the mean number of leaf spots per exposure period was highly correlated with both the modelled *R* and *R'* index values, with R^2 values from regression of 0.95 and 0.93 for the calculated *R'* index and the *R* index values respectively (Figure 5). For weeks 1 and 8, in which no symptoms developed, the weekly total of the risk model *R'* and *R* indices was zero. Control plants that were placed in the PC2

laboratory and not directly exposed to inoculum did not develop leaf spot symptoms. Control plants that were not directly exposed to inoculum from infected vines in block 18 but placed in the TPRO nursery, or TPRO weather station did become infected, but at lower rates of between 2.2 – 3.3 leaf spots per plant. A selection of typical leaf spot symptoms was polymerase chain reaction (PCR) tested on a leaf samples collected from trap plants from five different exposure periods and all were found to be of *Psa-V*.

Table 1. Dates of weekly ‘Hort-16A’ trap plant exposures to natural infection with *Pseudomonas syringae* pv. *actinidiae* (*Psa*) in Block 18 and dates for first appearance of leaf spot symptoms. The interval from likely infection date to symptom expression was estimated using the earliest day in each exposure week that field conditions appeared to be conducive to infection based on calculated *R* index value. Predicted *Psa* infection risk is shown as weekly total of the *R* index, calculated using wetness and days with >1.0 mm rainfall.

Week of exposure	Date of first symptoms	Interval from likely infection date to symptom expression	Weekly total of <i>R</i> index value	Total rainfall for days >1.0 mm (mm)
1 1/9-8/9	None	N/A	0	None
2 8/9 - 15/9	22/9	11 days	32	14.0
3 15/9 - 22/9	28/9	11 days	52	4.2
4 22/9-29/9	8/10	13 days	10	3.2
5 29/9 - 6/10	12/10	11 days	81	22.2
6 6/10 - 13/10	17/10	8 days	95	47.0
7 13/10 - 20/10	25/10	11 days	134	0.8
8 20/10 - 27/10	None	N/A	0	None
9 27/10 - 3/11	7/11	10 days	93	0.8
<i>Mean interval</i>		10.7 days		

The trap plants exposed in copper-treated plots developed significantly fewer spots per leaf ($P<0.05$) than those exposed in non-copper treated plots in weeks 4, 5, and 6 (Figure 2). In weeks 2 and 3, significantly more leaf spots developed on leaves from plants placed in the copper-treated plots ($P<0.05$). The effect of the previous foliar copper applications could not be evaluated in weeks 7 to 9 because there were not enough representative vines from each treatment remaining in the block to position the trap plants underneath. The trap plants were instead positioned under any remaining canopy.

In weeks 5,6,7 and 9 symptoms developed in plants exposed in the weather station that was not subject to inoculum directly from infected vines but rather from inoculum that was blown in from adjacent blocks at least 50 m or more in any direction. Similarly the control plants placed in the nursery area that is immediately adjacent to the weather station became infected in weeks 6, 7 and 8. The nursery contained no known infected vines and so was not considered to be a source of inoculum during these exposures. The fact that infection arose in control plants exposed around the weather station and the nursery area during the latter part of this study suggests that airborne Psa inoculum was present though-out the whole of the Te Puke Research Orchard.

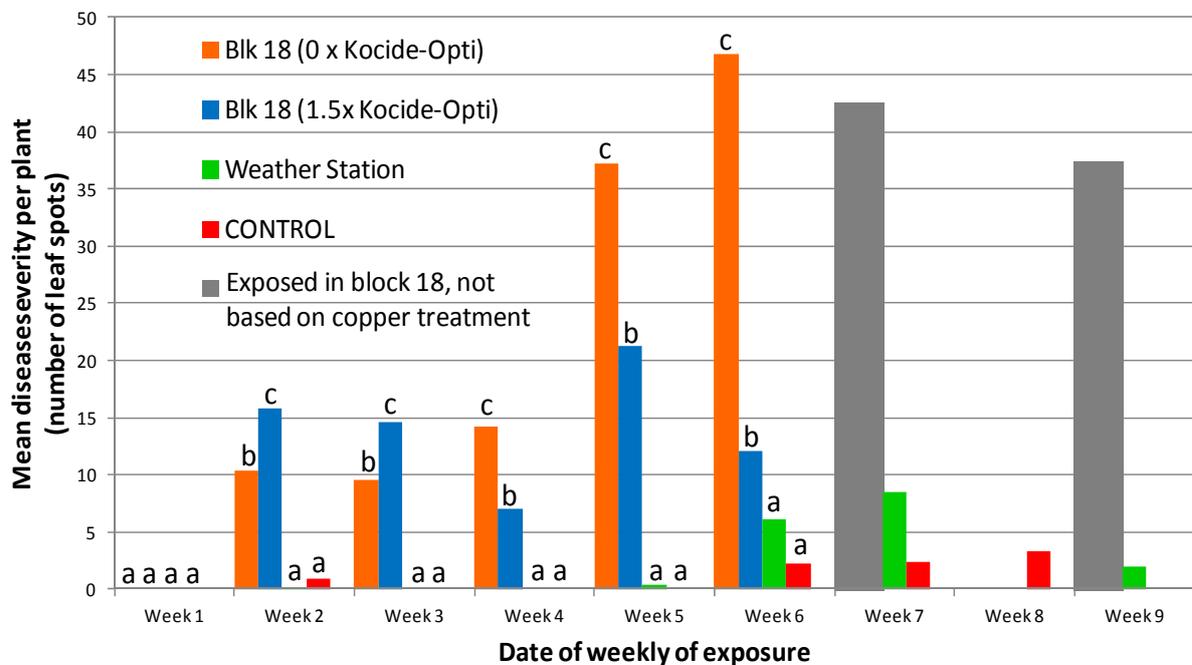


Figure 2. Psa leaf spot symptoms in ‘Hort-16A’ kiwifruit trap plants exposed for weekly intervals beneath the ‘Hort-16A’ canopy of Block 18 at Te Puke Research Orchard (TPRO). Trap plants were exposed in plots either without copper applications (0 gm/100L x Kocide-Opti) or with copper applications (120 gm/100L x Kocide-Opti) during leaf-fall and dormancy. Trap plant exposures began in early spring 1/9/2011 continuing to 3/11/2011. Additional trap plants were exposed at the TPRO weather station and control plants were maintained inside PC2-2 or in the nursery area of TPRO.

Relationship between predicted infection risk and trap plant leaf spot severity

Psa infection risk was calculated as both the daily R index using surface wetness and the R' index using RH. For 24 out of the 68 days during the trap plants exposure study, both the R and R' indices predicted infection risk (Figure 3).

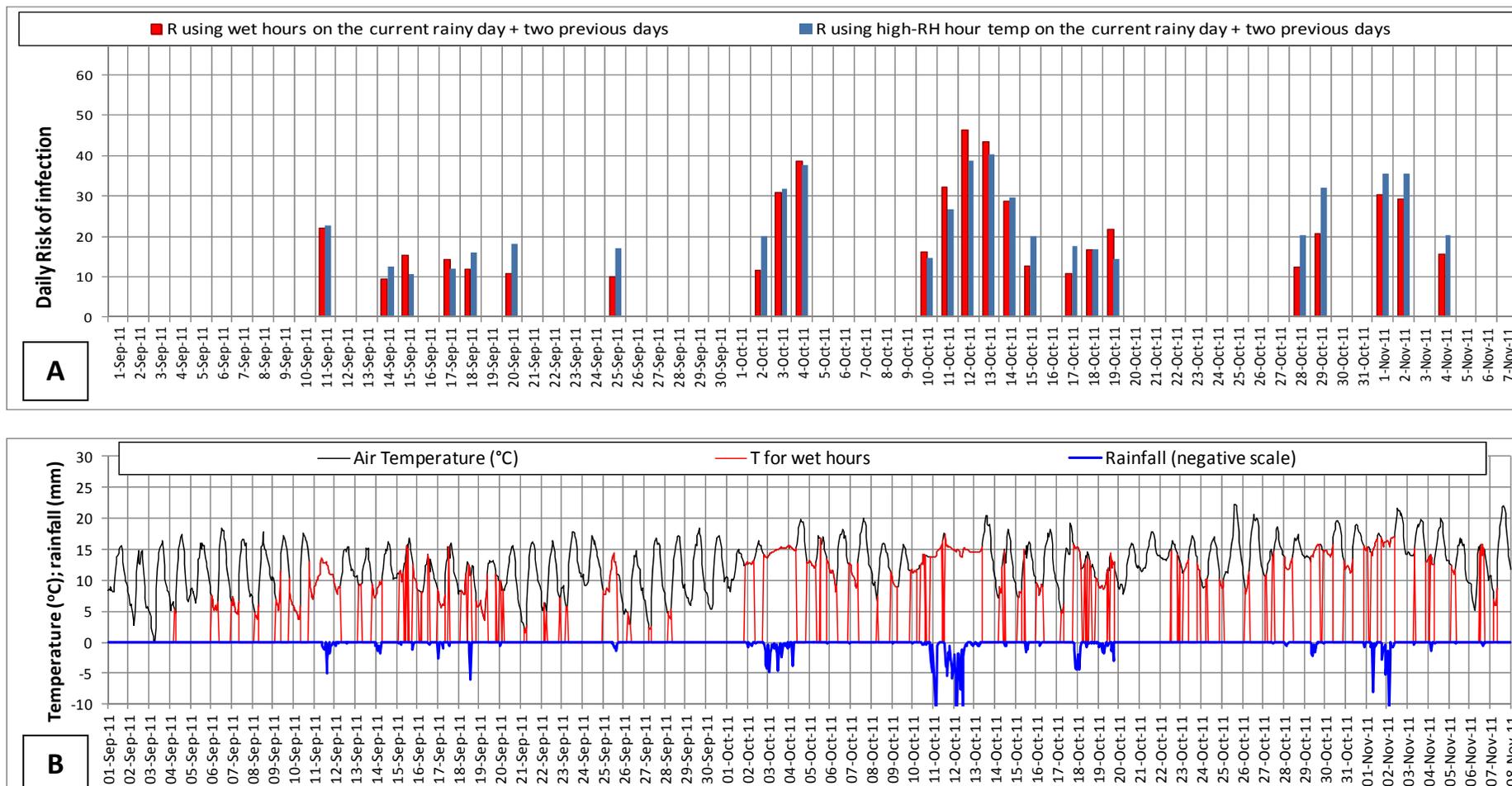
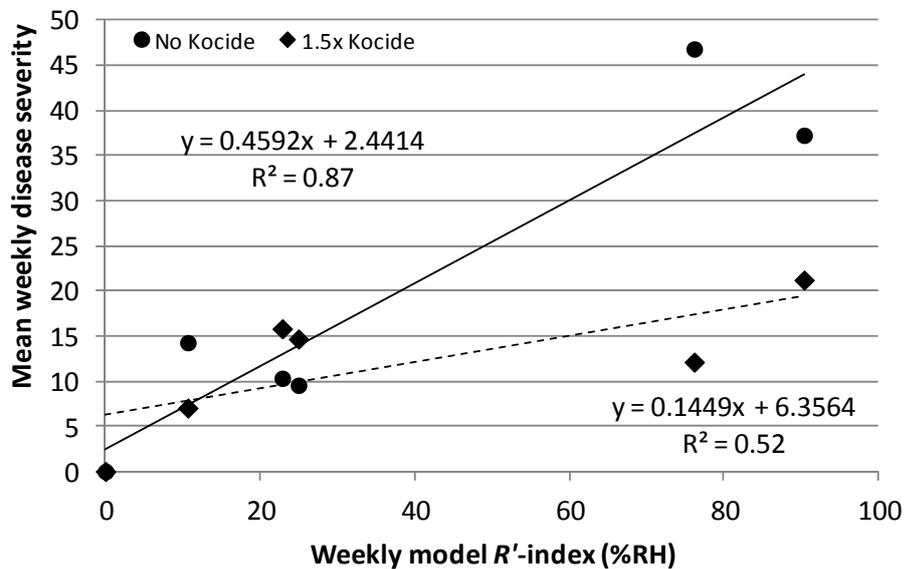


Figure 3. A) Daily output from the Psa infection risk model calculated from wetness (red bars) and RH (blue bars) and B) associated hourly temperature and rainfall during the 9 week trap plant exposure study in Block 18 at Te Puke Research Orchard. The red line in graph B indicates air temperature during wet periods. Rainfall (blue line) is plotted as negative values.

Although the effect of previous copper application on subsequent symptom expression was variable (Figure 2), when the weekly sum of the Psa infection R and R' index values was regressed on numbers of leaf spots from copper-exposed and non-copper-exposed trap plants separately, there was a significant difference in the regression slopes ($P < 0.05$, Figure 4a, 4b). This suggests that the winter copper sprays, which were applied 4-5 months earlier, had a lasting effect in reducing bacterial populations in the orchard. Additionally, the trap plants were exposed at ground level and the source of inoculum may have been a mixture of rainwater splash from within the canopy or from the soil and leaf litter at ground level. The high frequency of previous copper applications may have suppressed inoculum from both of these sources in the high copper treatment plots resulting in reduced symptom expression in trap plants.

R' index



R index

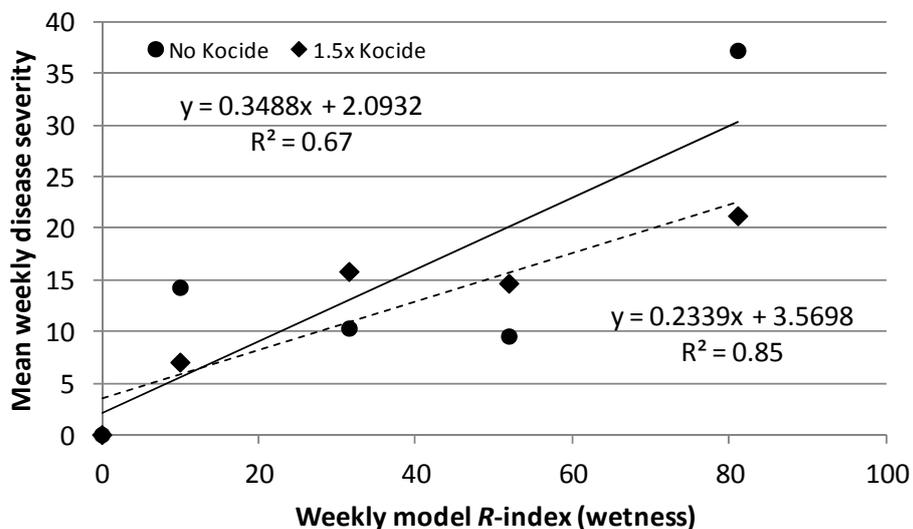


Figure 4. Regression of the weekly sum of predicted Psa infection risk using A) R' index calculated using percent relative humidity and B) R index calculated with leaf wetness, against trap plant disease severity, as mean number of spots per leaf, for separate copper-treated and non-copper-treated exposures. 'Hort-16A' trap plants were exposed beneath the 'Hort-16A' canopy of Block 18 according to prior copper application rate as ● Control (0 g/100L Kocide Opti) and ◆ 1.5 x Kocide Opti label rate (120 g/100L). Weekly trap plant exposures according to prior Kocide Opti application rate was conducted between 1/9/2011 – 13/10/2011.

Over all exposure weeks, the trap plants exposed under copper-treated vines showed significantly fewer lesions per leaf compared with those exposed under non-copper-treated vines. However, the effect of exposure to copper treatment was variable from week to week and was confounded in the last few exposure weeks by removal of infected vines from the orchard. Because the trap plants could not be exposed according to copper treatment over the entire 10-week experiment, trap plant data for copper and non-copper exposures were pooled and re-analysed. Regression of the pooled data showed that a high proportion of the variation in number of leaf spots was explained by both the R' index ($R^2 = 0.93$) and the R index ($R^2 = 0.95$) respectively (Figure 5).

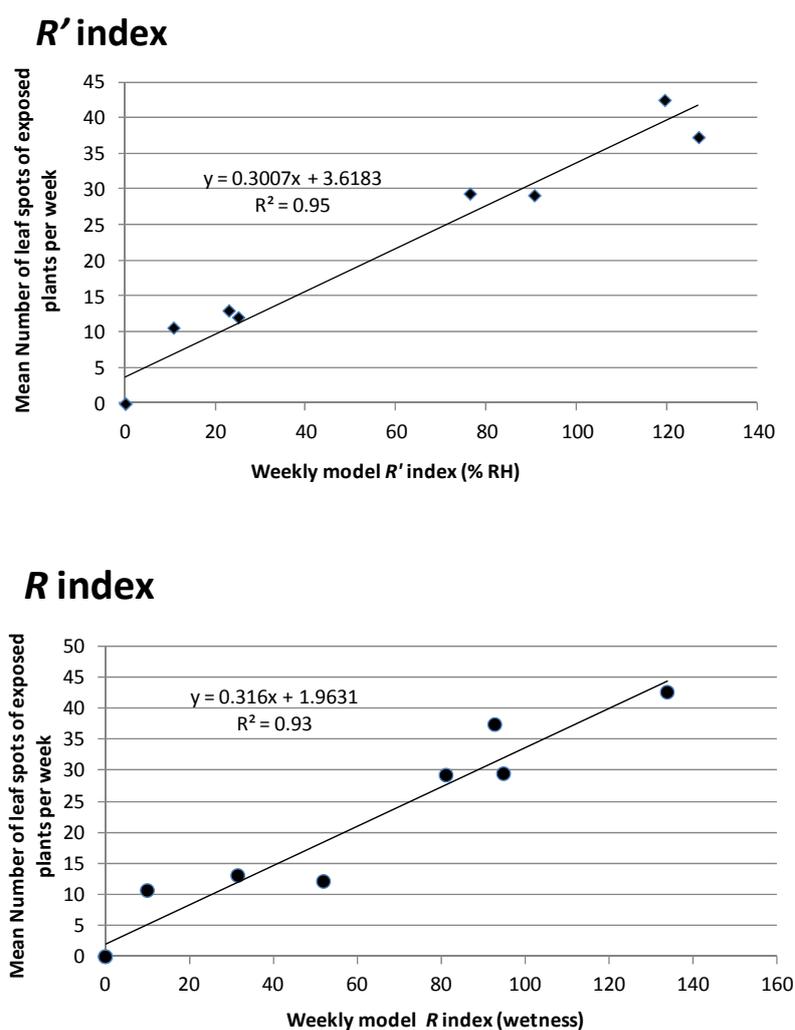


Figure 5. Regression of the weekly sum of predicted *Pseudomonas syringae* pv. *actinidiae* (Psa) infection risk on mean number of bacterial spots per leaf on nine sets of 'Hort-16A' trap plants, each exposed for one week then incubated for 20 days, using A) R' index calculated using relative humidity and B) R index calculated using leaf wetness. The number of leaf spots was averaged for trap plants exposed in copper-treated and non-copper-treated orchard plots.

CONCLUSIONS AND FUTURE RESEARCH STEPS

- This study using weekly exposures of trap plants in a kiwifruit orchard has shown that the preliminary Psa risk model developed by Plant & Food Research has very high prediction accuracy for kiwifruit leaf infection.
- This study shows that rainfall is the major weather variable driving Psa infection risk.
- Additional field and controlled-environment infection experiments are required to better define the environmental requirements for PSA infection so that the Psa risk model can be improved.
- Further research is required to identify conditions suitable for infection of kiwifruit canes and development of bacterial canker symptoms.

RECOMMENDATIONS FOR INDUSTRY

- Utilize PSA infection risk model to develop improved on-orchard PSA management strategies.
- Integrate the PSA risk infection model into existing weather forecasting and reporting systems to provide kiwifruit growers with information so that they can best optimise on-orchard PSA management strategies.

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Appendix 1. Psa risk model

A1.1 Bacterial multiplication index (M)

For each hour in which surface wetness or high relative humidity (RH) is recorded, the relative rate of bacterial multiplication (M) is given by the following equation:

$$M = -0.0000029 * T^4 - 0.00004 * T^3 + 0.00336 * T^2 + 0.0206 * T + 0.0153 \quad (1)$$

Where T is air temperature measured in a Stevenson screen. M has values between 0 and 1.

A1.2 Daily risk index using surface wetness(R)

The daily risk index, R , is evaluated at the end of each day (midnight) for days with more than 1.0 mm rainfall in the preceding 24 h. R is the sum of M values for hours during the current day and the two preceding days (potentially 72 hours) in which wetness was recorded. A wet hour is one in which the mean wetness sensor output was more than 50%. See below for estimation of R using high RH, rather than wetness. Evaluation of R prior to midnight on the current day requires forecast information for hourly temperature, wetness or humidity and rainfall. The maximum possible value of R is 72.

A1.3 Daily risk index using relative humidity (R')

Index M_{RH} is calculated from air temperature for each hour in which RH >94%. M_{RH} is calculated from Equation 1, as for M . Then, a daily risk index R_{RH} is calculated from the accumulation of M_{RH} for the current day and the two preceding days (potentially 72 hours). R_{RH} is evaluated at the end of each day (midnight), but only for days with more than 1.0 mm rainfall in the preceding 24 h. Evaluation of R_{RH} prior to midnight on the current day requires forecast information for hourly temperature, RH and rainfall. The maximum possible value of R_{RH} is 72.

Because M calculated for hours with RH >94% tends to identify more hours than M calculated for hours with wetness >50%, a correction to R_{RH} is required to give an estimate of R that is comparable with that calculated from wetness. This correction is given by the following equation between R (from wetness) and R_{RH} :

$$R' = 0.658 * R_{RH} + 0.0153 \quad (2)$$

R' is the fitted value from the regression of R_{RH} on R . Equation (2) explained 80% of the variation in R , over a 14 month period from August 2010 through September 2011 using weather data from Te Puke Research Orchard. The 94% RH threshold gave the greatest coefficient of determination ($R^2=80\%$) for the regression between R and R_{RH} when a range of RH thresholds was investigated. The mean absolute error in estimation of R from R_{RH} using the 94% RH threshold was 27%.

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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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