PLANT & FOOD RESEARCH *Pseudomonas syringae* pv. *actinidiae* (Psa) RESEARCH NOTE

KEY PROJECT DETAILS

Project Title	Psa Epidemiology – susceptibility of summer pruning wounds
Project Protocol No./ Objective No.	ZES VI1276 Pruning Wounds
Project Leader	Shirley Miller
Research Requested / Contracted by	ZESPRI
Date (Month, Year)	8 June 2012
Based on information as at	22 May 2012

RESEARCH QUESTION AND AIM

There is strong indication that the Psa bacteria can enter plants by colonising fresh pruning wounds. At this stage, there is little indication as to how long pruning wounds, or other sites of mechanical injury, remain susceptible to infection.

Determination of the importance of pruning wounds as infection sites will affect a number of management decisions, such as timing of pruning, and necessity to protect cuts.

This interim report describes an experiment carried out at Ruakura and Te Puke over the summer of 2011–2012 to assess the risks of summer pruning on 'Hort16A' vines.

METHODOLOGY (Include brief details of experimental design, methodology and protocols

Experimental design

Large, container-grown 'Hort16A' vines were used for the trial. There were 120 vines in total, with six pruning times x 20 replicates (where a single vine represented an experimental unit or replicate).

Methods and protocols

All plants were tested for Psa-V by Hill Laboratories Ltd before starting the trial; none was found to be positive.

Pruning treatments were imposed in early summer, starting 23 November 2011. At that stage, each plant had a main leader, and 3-5 vegetative side shoots. These vegetative side shoots were pruned at various time intervals before inoculation, using 20 plants on each occasion. There were six pruning events (64, 15, 7, 3, 1, or 0 days before inoculation) with each set of 20 vines pruned on a different day. Once all vines had been pruned, they were transferred to the PC2 laboratory at Te Puke for inoculation with Psa-V.

Inoculum was prepared from a fresh 24-h culture of Psa-V. Half the cut shoots on each plant were inoculated on 26 January 2012 by carefully applying a 10-µL droplet of bacteria (c. 10^9 cfu/mL) to the cut surface. The ends of the remaining cut shoots were inoculated with 10 µl saline solution. Thus each plant had both inoculated and non-inoculated shoots. Plants were left to dry for approximately 10 minutes before transferring directly to their field position in Block 1 West at Te Puke Research Centre. Some plants

received saline solution only. Pruning times on these plants were the same as previously described, but no Psa inoculum was applied.

Plants were monitored over the following 4 months for signs of infection (leaf spots, bud death, stunted growth and cankers). Assessments of inoculated and untreated control vines were made at regular intervals until the end of May 2012.

KEY RESULTS (all results must be auditable in terms of access to raw data if required)

The first signs of infection in this experiment were noted at the first assessment (2 March 2012), 5 weeks after inoculation, on shoots that had been inoculated on the same day as they had been pruned. Terminal buds were necrotic and there was a lack of leaf expansion. The shoots were clearly not healthy, and in contrast to shoots on all other plants, had failed to produce any new leaves since the day of inoculation 5 weeks earlier. The shoots then collapsed and died within the next 2 weeks.

Necrotic leaf spots typical of Psa had developed on inoculated and saline shoots for all pruning times when vines were assessed on 13 March, 7 weeks after inoculation. Necrosis of the terminal bud was often the first sign of infection and this was significantly worse in the inoculated shoots (P=0.028). Terminal bud death was sometimes but not always accompanied by leaf spotting. Once started, symptoms developed quickly and appeared to be moving progressively along a shoot and into un-inoculated shoots. This observation was supported up by isolations from shoots dissected into short lengths and identification of bacteria using PCR.

The background incidence of bacteria was high at Te Puke, and although the plants were healthy and free from Psa-V at the start of the trial, nearly all the un-inoculated control vines showed leaf spot symptoms after 4 months, at the end of May. Nevertheless, at the earlier assessment date on 13 March, the disease severity rating for control shoots was significantly less (P<0.001) than for inoculated shoots (Figure 1), meaning that the inoculated shoots were more likely to be infected than controls. Although fresh pruning cuts or those made 24 hours before inoculation were infected most rapidly, older cuts also became infected and after 7 weeks there were no significant day effects. The trend line in Figure 1 suggested that severity of the infection appeared to be greatest in vines pruned 15 days before inoculation.

Displaying the data on the logit scale showed that as the number of days between pruning and inoculation increased up to 15 days, the probability that shoots remained healthy decreased. When the time between pruning and inoculation was 64 days, there was a greater probability that the shoots would still be healthy after 7 weeks (P=0.006). Seven weeks after inoculation, inoculated shoots had about a 0.15 lower probability of being healthy than uninoculated shoots (Figure 2).

Secondary symptoms (bud death, collapsed shoots, wilting and cankers) were more likely to occur on inoculated shoots.

Whole-vine assessments on 22 May showed a similar trend to shoot data, with a tendency for increasing severity in disease expression in vines pruned up to 15 days before inoculation (Figure 3). There was insufficient information for the data to be further analysed.

Data Analysis: Link to statistical analysis is http://biodev3.pfr.co.nz/bdw/index.php/Psa_epidemiology

There are two datasets from two different Psa evaluation times, using two different disease severity rating scales, assessed either for shoots or whole vines:

- 13 March 2012 (inoculated v. un-inoculated shoots)
- 22 May 2012 (whole vines: un-inoculated controls compared with inoculated plants)

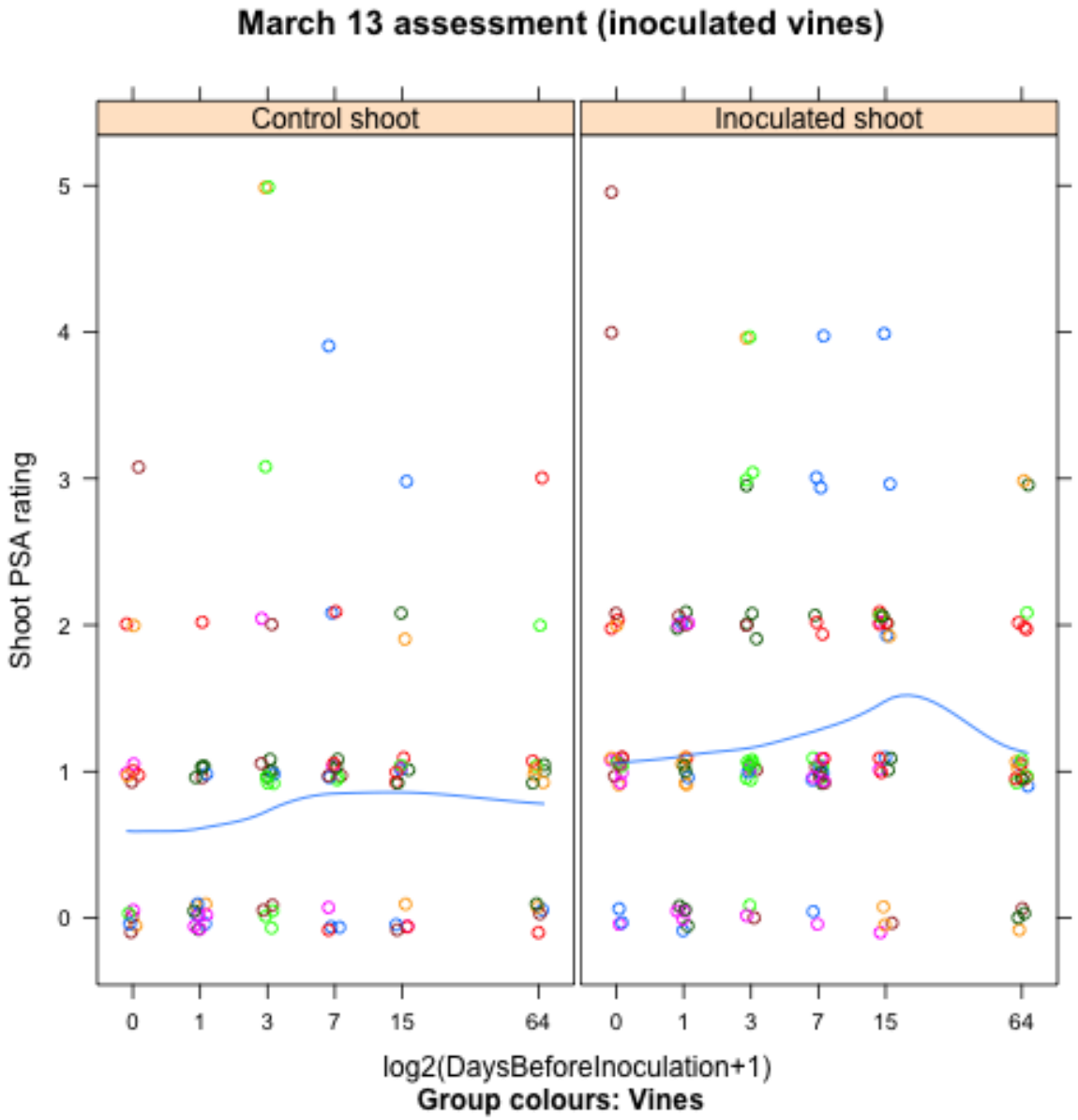

Figure 1. Severity rating for un-inoculated control 'Hort16A' shoots (left) compared with shoots inoculated with Psa, assessed on 13 March 2012, 7 weeks after inoculation. Background infection has been taken into account in the analysis.

The loess curves suggest that inoculated shoots have higher scores than control shoots, and that the incidence score of Psa symptoms was maximised for shoots pruned 15 days before inoculation.

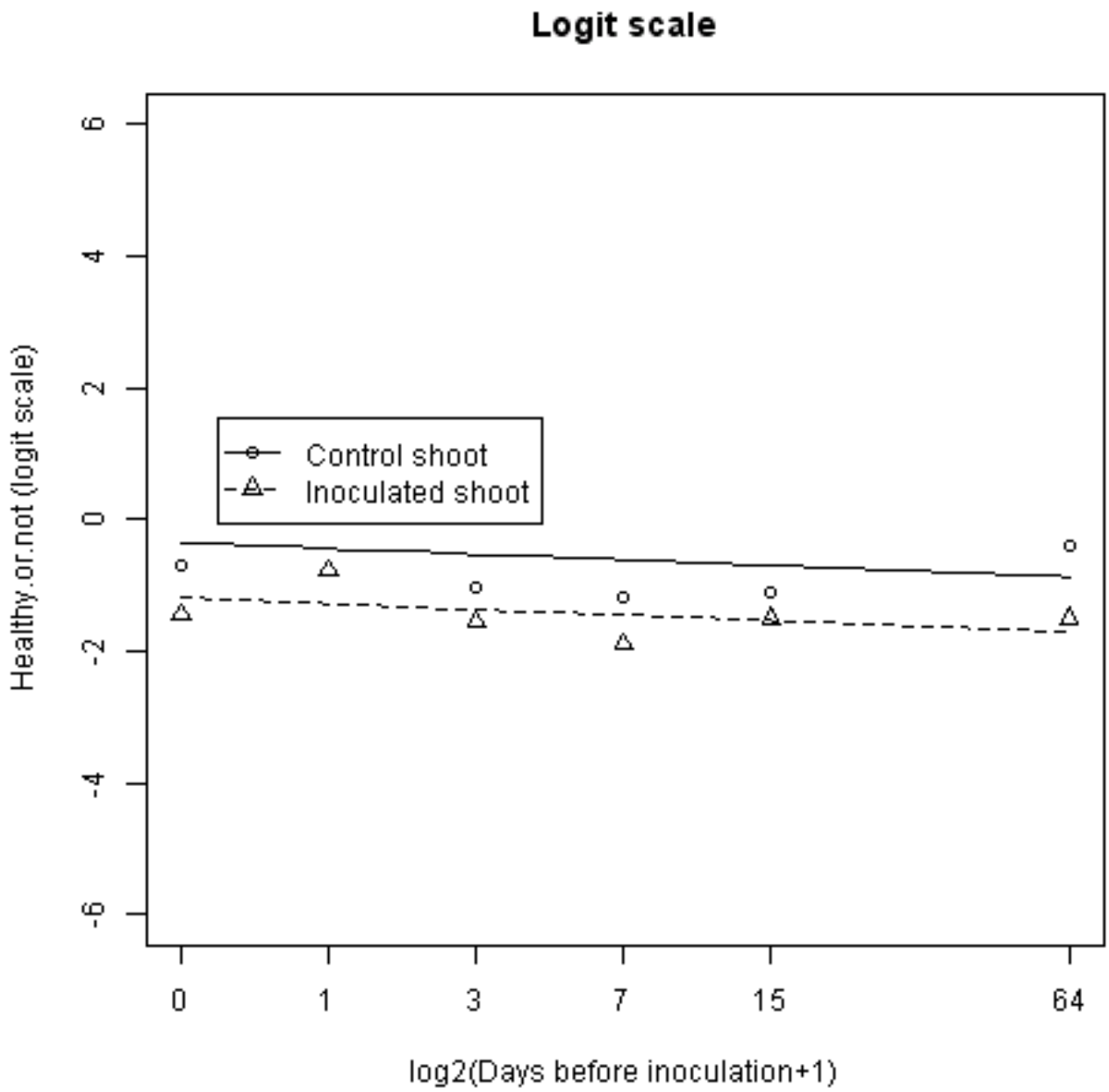

Figure 2: Logit scale plot to show decreased probability of healthy 'Hort16A' shoots when inoculated with Psa 0-64 days after pruning, compared with control shoots pruned at the same time intervals (7 weeks of assessment).

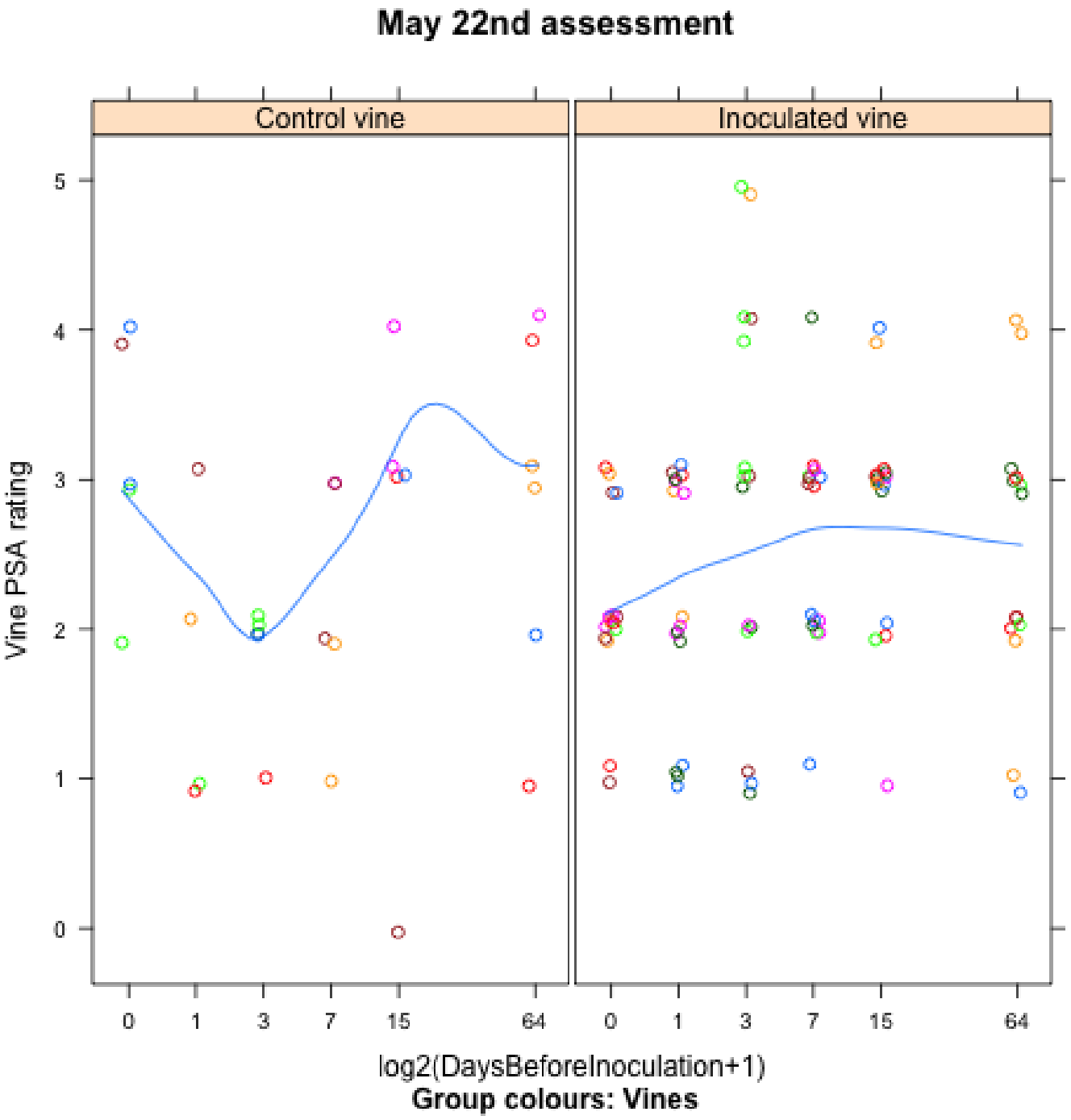

Figure 3: Severity rating for un-inoculated 'Hort16A' control vines (left) compared with vines inoculated with Psa after 4 months (assessed 22 May 2012).

RECOMMENDATIONS FOR INDUSTRY

Most cuts became infected in this trial, regardless of time between pruning and inoculation (0-64 days).

Key points:

- Summer pruning is risky, and wound healing will be delayed if carried out under cool, wet conditions.
- Pruning cuts need immediate protection with antibacterial agents.
- Products that promote rapid healing of wounded tissue would be worthwhile investigating.

DISCUSSION

Exploratory data plots appeared confusing in that they inferred higher disease severity ratings on vines pruned 15 days before inoculation. Intuitively it might be assumed that lower numbers of days would be worst, with fresh wounds most susceptible to infection. This assumption is supported by the observation that bacteria appeared to be absorbed into the plant's vascular system most rapidly when inoculum was applied to a freshly cut surface. In contrast, a bubble of liquid remained suspended above the cut stem for at least 10 minutes when applied to cuts made 64 days before inoculation. It is known that wound healing is affected by weather conditions (i.e. rainfall and temperature). A total of 12 mm of rain fell on 5 days out of the 15 before inoculation and this may have affected the healing process for that set of vines. Similar work with infection of trunk girdling wounds showed that bacteria can enter via fresh callus (Snelgar et al 2012), so callusing may also be a reason why plants pruned 15 days before inoculation were quite susceptible compared to day 0. At day 0 there would be lots of wound response phenolics which could temporarily inhibit the bacteria but that would disappear as new callus cells formed. Thus early stages of wound healing could in fact be more beneficial for the bacteria than the plant.

An alternative explanation, or compounding effect, could be that high concentrations of bacteria were applied to a cut surface where healing might have been starting to occur but was not yet complete (e.g. 15 days after pruning). At this time, bacteria were not be able to invade immediately and multiplied in the interim, accumulated to even higher numbers, and eventually infected the tissues, causing disease symptoms that were worse than if the bacteria had been absorbed immediately.

At 64 days before inoculation, even though there was a total of 322 mm of rain, the healing process was more complete, and many of the bacteria may have died before infection occurred, hence the lower disease severity rating for these plants.

CONCLUSIONS

All summer pruning wounds at least up to an age of 2 months are potentially susceptible to Psa infection.

FUTURE RESEARCH STEPS

Further investigation into the wound healing process in kiwifruit is needed, particularly enzymatic reactions and histological changes in relation to rainfall and temperature. Integrating the influence of environmental factors will help to better understand the interaction with bacterial invasion.

A similar trial to that described is planned for winter pruning cuts, using dormant vines.

If, as was suggested by the results in the summer pruning trial, effects are worse under slow wound healing conditions and/or when bacteria are allowed time to accumulate on a surface before being able to breach defence barriers, then the application of products that accelerate the healing process in the vine would be worth developing.

REFERENCES

Snelgar, B., Blattmann, P., Tyson, J., Curtis, C., and Manning, M. (2012) Girdles can be infected with Psa-V New Zealand Journal of Kiwifruit – Psa Scientific Edition. May/June 20-23.

ACKNOWLEDGEMENTS

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DISCLAIMER

LIMITED PROTECTION

CONFIDENTIALITY

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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