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

KEY PROJECT DETAILS

Project title	**VI1279 Bioassay evaluation of vacuum infiltration of systemic bactericides against Psa infection of woody stems**
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QUESTION

Systemic injection of anti-bacterial products may have the ability to reduce *Pseudomonas syringae* pv *actinidiae* (Psa) disease progress between vines within infected orchards and within individual vines. This project is aimed at assessing an existing bioassay, developed for screening different genotypes for tolerance to Psa, as a potential tool for screening systemic bactericides.

RECOMMENDATIONS

- As a proof of concept study, the woody stem bioassay has demonstrated it does have potential for screening Psa antagonistic products when they are vacuum infiltrated into woody canes.
- We recommend that cane samples should be collected from whole vines in orchards which have been treated with KeyStrepto® by injection into the vascular system, so that the possible confounding aspect of vacuum infiltration (sap replacement) can be reduced.
- Other treatment technologies (such as bacteriophages) or products (e.g. Bion®) with potential to suppress Psa bacteria should be evaluated using this bioassay, following vacuum infiltration or whole vine injection.

AIM

To determine whether a woody stem bioassay, developed for screening tolerance of kiwifruit to Psa, can be used to screen the activity of systemic bactericides.

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

- A preliminary study was carried out prior to the main experiment to check that vacuum infiltration of a coloured dye into cane segments resulted in successful transfer of liquid through the length of cane segment.
- A 1 m length of Hort16A cane, which had been collected from dormant vines at Ruakura and stored in sealed plastic bags in a cold store, was cut into several 10–50 cm segments and placed in water containing aniline blue dye. Tubing was attached to the top end of the cane segments, sealed and a vacuum applied to draw the liquid through the cane segment.
- For the main experiment, six Hort16A kiwifruit canes were removed from the cold store and each was

cut into six 10 cm segments. Two additional canes were also collected directly from vines at Ruakura which had undergone bud-burst.

- Cane segments from individual canes (removed from cold store) were assigned to treatments 1–6 (six replicates per treatment) and cane segments from the canes removed from the field were assigned to treatments 7 & 8.

Treatment List:

Treatment No.	Treatment description
1	Dry control
2	Dry control (+ Psa)
3	SDW infiltration (+ Psa)
4	100 ppm streptomycin infiltration (+ Psa)
5	200 ppm streptomycin infiltration (+ Psa)
6	400 ppm streptomycin infiltration (+ Psa)
7	Dry control (fresh canes) (+ Psa)
8	100 ppm streptomycin infiltration (fresh canes) (+ Psa)

- Streptomycin was prepared at the required concentrations from a stock solution containing 1 g streptomycin sulphate in 10 mL water.
- Vacuum apparatus was connected to cane segments and the appropriate treatment solutions were drawn up through the canes until excess liquid exuded from the top end of the cane.
- An aliquot of the exudate was collected for estimation of the streptomycin content using a bacterial over-lay plate method. For this, Petri dishes of bacteriological agar were overlaid with a thin layer of soft agar to which Psa bacteria had been added. Aliquots (10 μL) of the exudate collected from each infiltration treatment (five replicates per treatment) was placed onto these overlay plates. Similarly, aliquots (10 μL) of a range of concentrations (1, 2, 5, 10, 20 and 100 ppm) of KeyStrepto were placed onto the same overlay plates, so that a comparison in inhibition zone could be made between the known concentrations of streptomycin and the exudate samples.
- Cane segments for the main experiment were then taken to a PC1 laboratory and laid into plastic trays.
- Two wounds were marked on each cane segment and 10 μL of freshly prepared Psa inoculum (>1 x 10^9 cells per mL) was placed directly into the fresh wound site.
- Cane segments were incubated in the laboratory. After two weeks one of the inoculation points on each cane segment was assessed for symptoms (lesion) and signs (bacterial ooze). The bark adjacent to each wound site was carefully shaved back using a Stanley® knife blade to expose the lesion.
- The full length of the exposed lesion was measured using digital callipers and then the trays were closed. The following day each wound site and lesion was observed using a binocular microscope and the location and quantity of bacterial ooze in the wound site and on the exposed lesion was scored on

a 0–4 scale. After three weeks incubation, the other wound was similarly assessed.

- Lesion size and ooze score data for the two wounds on each cane segment were averaged and then analysed by ANOVA using Genstat (10th Edition). In addition, a woody stem bioassay index (with scale 0-100) was calculated by combining lesion size and ooze score (with equal weighting). This was also analysed by ANOVA using Genstat.

KEY RESULTS

Preliminary study

- The aniline blue dye was observed to be readily drawn through the cane segments.

Main experiment

Lesion size

- The mean lesion length in the dry control (– Psa) (2 mm) represents the width of the wound when no infection takes places (Figure 1).
- The mean lesion length in the two dry controls (+ Psa) and the SDW control ranged from 16 to 20 mm and these were not significantly different to each other.
- The mean lesion length in the 100 ppm streptomycin treatment (14 mm) was not significantly ($P>0.05$) less than the dry and SDW controls.
- The 200 and 400 ppm streptomycin treatments (8–11 mm) were significantly ($P<0.05$) less than the SDW control and the 400 ppm streptomycin treatment was also significantly less than the dry control (Figure 1). However, even in the 400 ppm streptomycin treatment there was only about 50% reduction in lesion size compared with the dry and SDW controls.
- There was a significant trend of reducing lesion length with increasing concentration of streptomycin.
- The 100 ppm streptomycin treatment using fresh canes had similar lesion length as the 100 ppm streptomycin treatment using cold stored canes and was significantly less than the dry control using fresh canes.

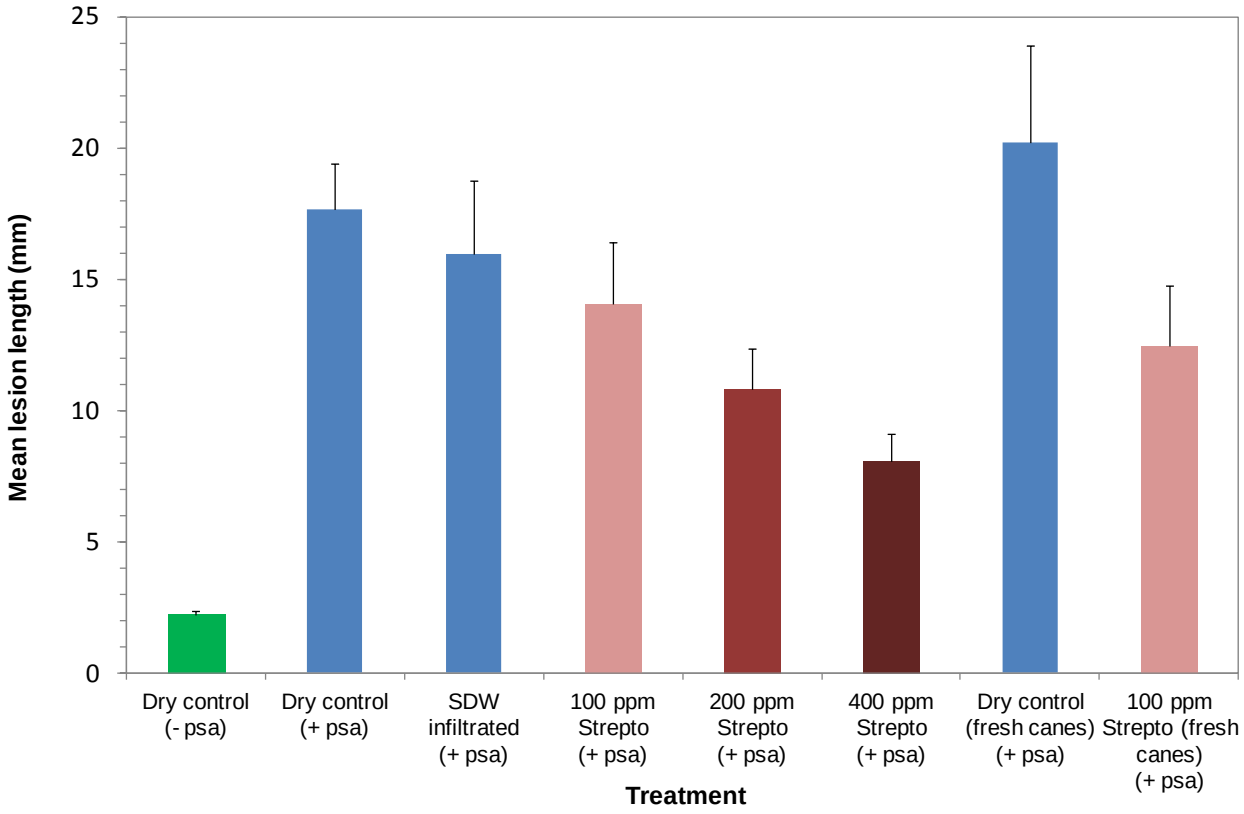

Figure 1. Mean lesion length on woody canes following infiltration with SDW and three rates of streptomycin and inoculation with Psa in freshly cut wounds. Least significant difference (LSD) = 6.8 mm and bars are standard error of the mean.
Where: SDW = Sterile distilled water and Strepto = Streptomycin.

Ooze score

- The mean ooze score ranged from 2.3 in the 100 ppm streptomycin treatment using fresh canes to 3.0 in the SDW control and 200 ppm streptomycin treatment. There was no significant treatment effect on the mean ooze score (Figure 2).
- There was relatively profuse ooze development on several of the canes in this experiment, including in the streptomycin treatments, compared to what has been observed during genotype screening where canes are not vacuum infiltrated with liquid.

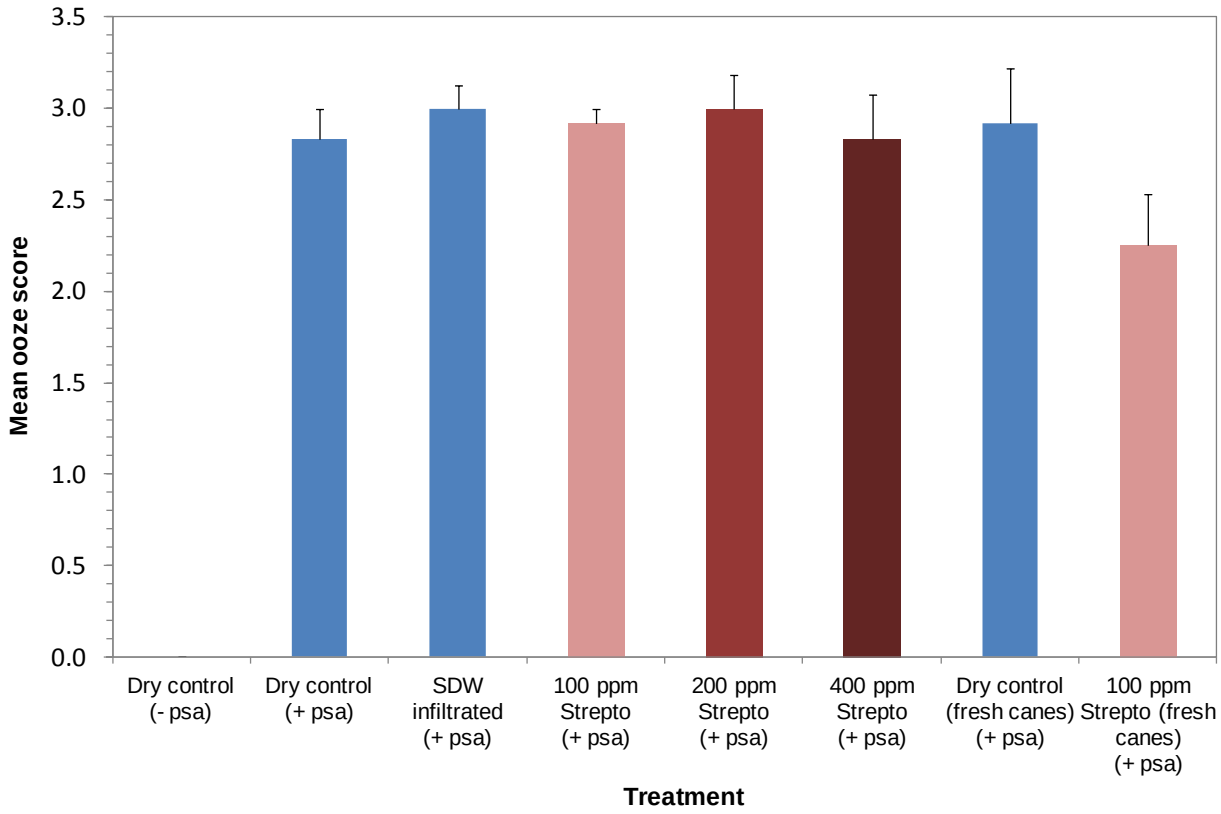

Figure 2. Mean ooze score on woody canes following infiltration with SDW and three rates of streptomycin and inoculation with Psa in freshly cut wounds. Bars are standard error of the mean.
Where: SDW = sterile distilled water and Strepto = streptomycin.

Woody stem bioassay index

- The calculated woody stem bioassay index is shown in Figure 3 and ranged from 62–78 across all treatments.
- There was a trend of reducing index value with increase in the rate of streptomycin, such that the 400 ppm streptomycin treatment was significantly ($P<0.05$) lower than the dry and SDW controls.
- The woody stem bioassay index values in this experiment were very similar to 'Hort16A' in similar bioassays carried out to investigate susceptibility of kiwifruit genotypes to Psa infection (Figure 3). For this experiment the four streptomycin treatments had higher index values than untreated woody stems of 'Hayward'.

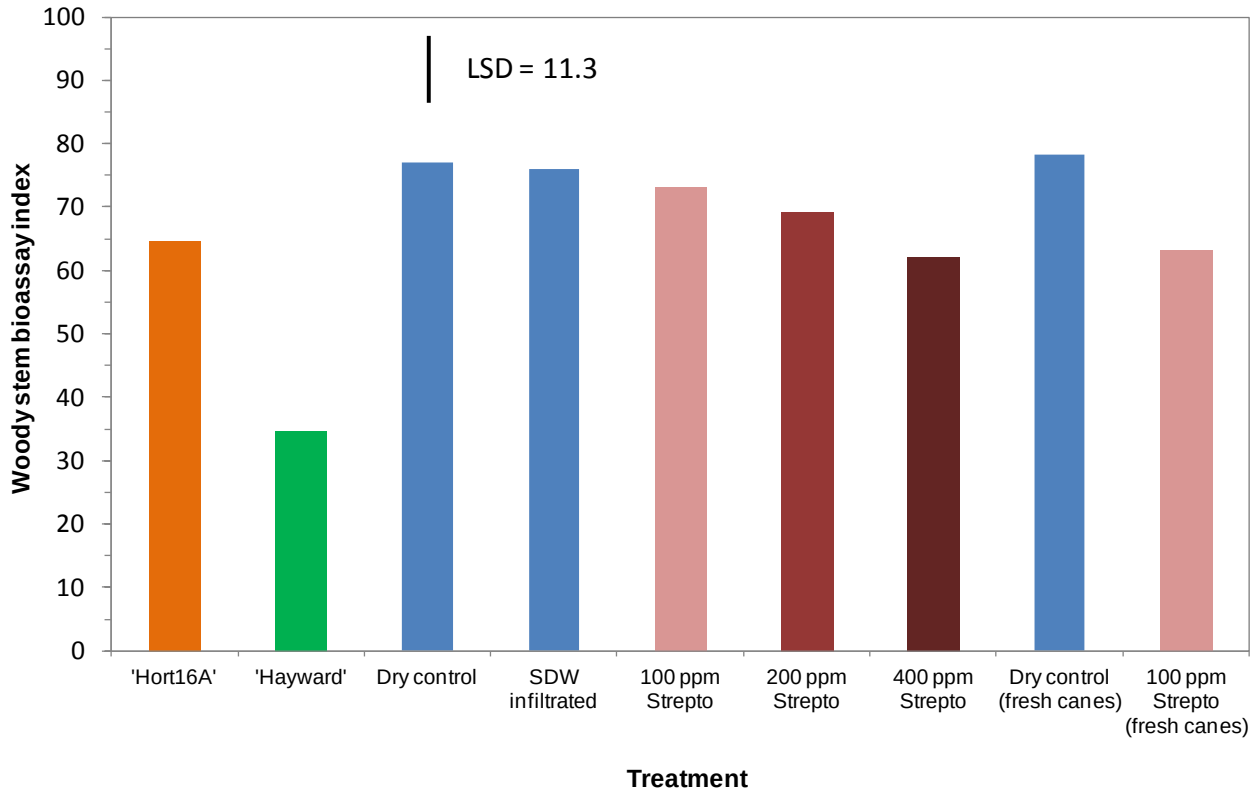

Figure 3. Mean woody stem bioassay index (combined lesion size and ooze score) for woody canes following infiltration with SDW and three rates of streptomycin and inoculation with Psa in freshly cut wounds. Data for 'Hort16A' and 'Hayward' is taken from PFR genotype screening research programme and is the mean of 20 separate bioassays. Least significant difference for streptomycin treatments (LSD) = 11.3.
Where: SDW = sterile distilled water and Strepto = streptomycin.

Streptomycin content of exudate

- The mean inhibition zones for the range in concentrations of KeyStrepto is shown by the red line plot in Figure 4 and ranged from 0–16.6 mm.
- The mean inhibition zone for Treatments 4, 5, 6 and 8 were 0.8, 4.2, 8.2 and 4.4 mm respectively (shown on the y-axis, Figure 4).
- The equivalent KeyStrepto concentration (by linear extrapolation) for the exudate from these four treatments was approximately 2 ppm for T4, 6.5 ppm for T5, 18 ppm for T6 and 7 ppm for T8. Hence there was an increase in the effective concentration of streptomycin in the exudate from the three treatments with increasing streptomycin concentration (T4, T5 and T6), but the concentration in the exudate was 1/20th to 1/50th of that in the initial treatment solution infiltrated into the cane segments.
- Therefore, it appears that the effective concentration of streptomycin in T8 (100 ppm streptomycin in fresh canes) was higher than in T4 (100 ppm streptomycin in cold stored canes) and this may be due to the different physiological state of these two cane samples.

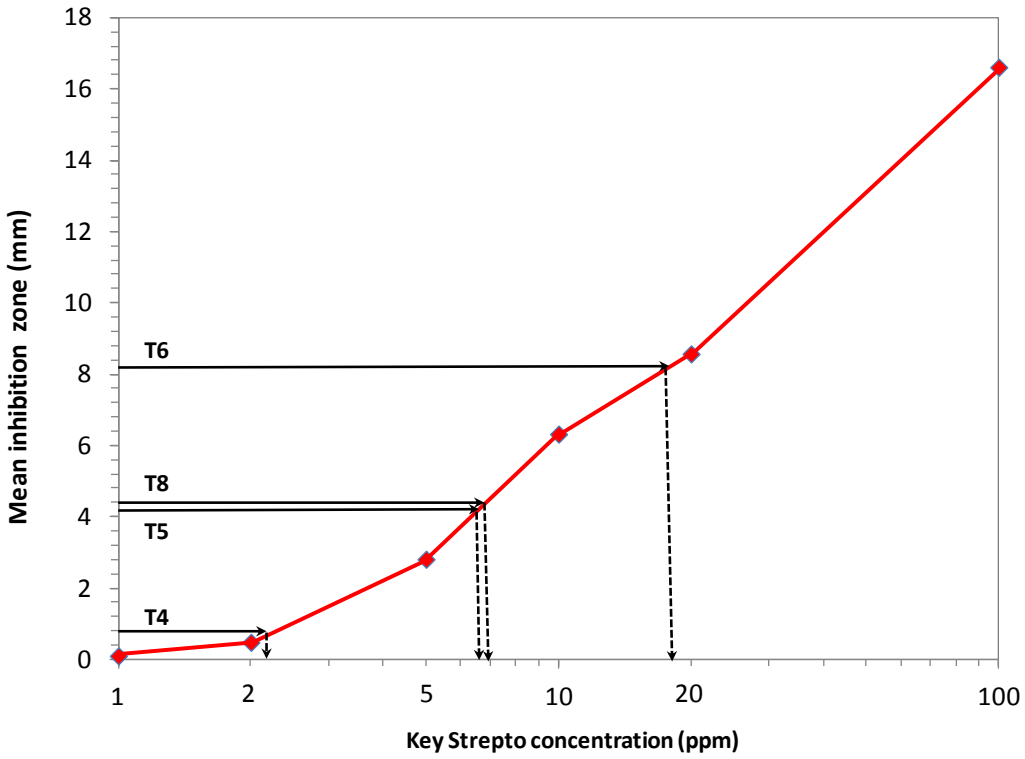

Figure 4. Mean inhibition zone (mm diameter) on Psa overlay plates treated with 10 µL droplets of a range of KeyStrepto® concentrations and with exudate collected from four of the infiltration treatments. Note the x-axis is on a log scale. Where T4, T5, T6 and T8 are the mean inhibition zone diameters for exudate from treatments 4, 5, 6 and 8 respectively. Dashed line represents the linear extrapolation of exudate results onto the KeyStrepto concentration scale.

DISCUSSION

- The woody stem bioassay (that has been developed for screening kiwifruit genotypes), detected suppressive effects of streptomycin on Psa infection when vacuum infiltrated into cane segments, and therefore the bioassay may be a suitable tool to screen systemic bactericides.
- Lesion size was only reduced by 50% in the 400 ppm streptomycin treatment, indicating that complete inhibition of Psa infection was not achieved.
- The estimated equivalent concentration of streptomycin in the exudate collected from the ends of canes showed that the concentration of streptomycin was substantially lower than in the solution being infiltrated, indicating dilution and/or neutralisation by the plant sap.