

Pre-flowering Budrot Protection

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Executive Summary

Pre-Flowering Budrot Protection

June 2014

This project had 3 goals:

1. To assess the efficacy of Psa sprays against pre-flowering budrot.
2. To determine whether the recently observed budrot was caused by Psa or other bacteria.
3. To record any plant effects of the new Psa spray Ambitious®.

The site had been severely affected by budrot in the previous season and this was reflected in variability across the block both visually and in our assessments

Ambitious® effects

1. Ambitious appeared to effect the plant process which was occurring at the time of its application. Consequently different effects were observed on different orchards and on different varieties. Commonly observed effects included
 - a. Redness on developing leaves and stems
 - b. Larger diameter (but sometimes shorter) fruit stalks and larger diameter leaf petioles
 - c. Thicker leaves
 - d. Larger hanging buds
 - e. Slightly extended flowering with more later and deformed flowers
 - f. Hairier fruit which was sometimes gingery in colour
 - g. Water loss was higher from these “ginga” fruit than from visually unaffected fruit
 - h. At this stage we have not seen consistent increases in fruit size
 - i. We have not seen increased water loss in storage.

Budrot

2. The observed “budrot” was not associated with wet rotting. In all cases, and through wet weather, the buds stalks and flowers were simply brown and, in severe cases, desiccated.
 - a. Browned flowers and buds remained long after flowering and when they finally dropped the stalk persist even longer as a thin brown thread.

Microbiology

3. We have characterised the plate morphology of several Psa strains, *P viridiflava*, and *P sp* on several media which provide distinguishing characteristics for positive identification.
4. Partial sequencing of the 2 strains originally associated with budrot raised more questions than answers – they did not match any sequences in Genbank
5. None of the strains of Psa showed any copper or streptomycin resistance which should mean that current Psa spray programmes should have efficacy against these bacteria
6. The *Pseudomonas sp (viridiflava)* showed resistance to copper at Cu = 0.32 mM and streptomycin at 1µg/mL.
7. Psa-V and *P sp* were grown on the underside of HW leaves and populations flourished over 7-11 days without any plant symptoms becoming evident. Population trends showed that *Pseudomonas sp (viridiflava)* grows faster than Psa-V to reach a maximum



≈48 hrs post inoculation. Psa-V took ≈5 days to reach its maximum population under the same conditions.

8. Both HW and G14 samples collected pre- and post-treatments had high Psa counts with no traces of the other 2 budrot associated bacteria.

Bacterial cause of Psa

9. In all cases from slight browning of sepals through to complete desiccation of the hanging buds we found very high numbers of Psa.
10. Conversely we rarely found high Psa numbers on buds with no browning.
 - a. Even if Psa is responsible for browning we might expect to find occasionally normal buds with Psa populations if the bacteria had only recently arrived on the surface.
11. We did not find evidence of other bacteria traditionally associated with budrot.
 - a. We acknowledge that in cases of multiple bacteria in very unequal proportions it may be very hard to find those of very low numbers
12. The observed “budrot” was not associated with wet rotting. In all cases, and through wet weather, the buds stalks and flowers simply brown and desiccate.
 - a. Browning flowers and buds remained long after flowering and when they finally dropped the stalk persist even longer as a thin brown thread.
13. The rainfall and KVH risk index showed significant risk associated with the rainfall in the period from the 19-25th Sep which was 12 days prior to the spotting we observed.
14. After periods of rainfall (Figure 20) buds could be found with white deposits remaining on the drip point (Figure 50). These may well be associated with Psa as very similar material has been collected previously and found to contain high levels of Psa.

Spray efficacy

15. The site had been severely affected by budrot in the previous season and this was reflected in variability across the block both visually and in our assessments.
16. Our experience over the first few days showed
 - a. Very rapid development from no symptoms to extreme symptoms
 - b. Areas of bud rot were seemingly scattered randomly over the block although there appeared to be more in the lower areas where damage had been worse in the previous season.
 - c. Symptoms were not assessed in relation to variety of male they were adjacent to. In the previous year males, especially M91, succumbed to Psa leaving more M33 in the block and significant numbers of missing vines in the male rows.
 - d. These symptoms stopped spreading when we appeared to be on the brink of major widespread budrot.
 - e. The spread, and cessation of spread, occurred before the treatments in this trial were applied.
 - f. We are puzzled by the rapid cessation of symptom development since weather conditions were cool and wet and it occurred even on our control plots which received no treatment.
17. This rapid spread of Psa symptoms immediately prior to the trial being laid down occurred 12 days after significant rainfall when the KVH risk index was high (Figure 6).
 - a. It remains a puzzle as to why the symptoms ceased to spread this season since at this time (7th Oct onwards) we again entered a period of high risk.
 - b. It is also a puzzle why the previous season had such a serious loss since it had significantly less rain (and consequently risk) than we experienced this season (Fig 6).



18. Vines were assessed for the extent and severity of leaf spotting as well as for bud browning
 - a. The extent of spotting was worse at the ends of canes adjacent to male rows
 - b. This trend was less obvious for severity of spotting and not apparent in relation to bud browning
 - c. We did not assess symptoms in relation to local canopy density.
 - d. The extent and severity of spotting was not related to Psa treatment
 - i. But it did vary by position (row) across the block) i.e. we hypothesise it was affected by microclimate and perhaps previous infection levels (presumably also affected by microclimate)
 - e. Bud browning was related to treatment (Table 7) and varied from 5.7 to 21.3%.
 - i. These losses may have been underestimated because of the way they were characterised although the fact that cosmetic loss was not related to treatment increases our confidence in the validity of our measurements.
19. The variability in symptoms day-to-day suggests future trials should endeavour to assess efficacy more frequently after treatment application.
 - a. The relative efficacy of treatments may become more apparent if this can be undertaken

Pre-flowering Budrot Protection October 2013

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Introduction

The most dramatic effect of the bacterial canker caused by *Pseudomonas syringae actinidae* (Psa) was the rapid decline and death of the Hort16A kiwifruit variety. Subsequently the effects of the bacteria spread to the other commercial kiwifruit varieties including the most resistant of the recently released Zespri varieties (G14) and to Hayward (HW) which is the original variety on which the industry was based and is still heavily dependent.

Psa symptoms vary between varieties. Whereas H16A first exhibited leaf spotting (primary symptoms) which typically and rapidly progressed to shoot wilt, extensive oozing (secondary symptoms) and finally vine death, neither HW nor G14 have followed this progression. Leaf spotting is common on these varieties as is some oozing, but wilting is rare. What has been observed on both varieties in some regions, is extensive “budrot” prior to flowering leading to yield reductions of up to 70%. Last season KVH undertook a survey of G14 orchards and found the levels of budrot shown in Figure 1.

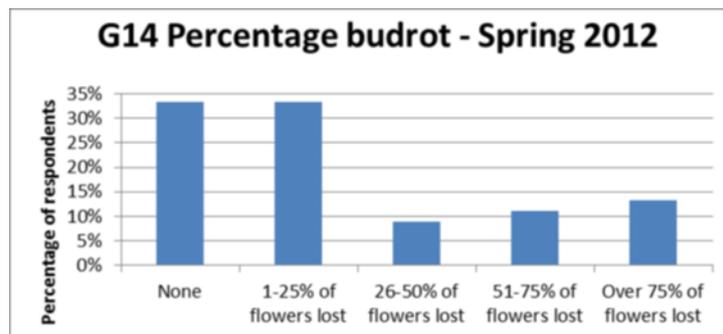


Figure 1. KVH G14 budrot survey results.

While budrot has been present for many years prior to the advent of Psa it has always been highly variable both seasonally and regionally however, the significant levels of bud loss following the arrival of Psa begs the question as to whether Psa is now implicated in causing or exacerbating this problem. Losses have been particularly severe to the East of Te Puke - on the flats and hills towards the coast - and also further south in Edgcombe. These areas are characterised by shallow and wet soils, cold winters and often late-winter frosts. We also recognise the potentially overriding effect of seasonal weather. Have we simply had weather sympathetic to budrot for the last 2 winters in the affected areas, or is Psa contributing to, or causing, these widespread symptoms? If Psa is implicated do current Psa sprays also prevent or ameliorate budrot?

We use the term “budrot” in this report but we are aware that there is significant uncertainty among pathologists as to the casual bacteria and even the symptoms covered by this umbrella term. Budrot was first reported in 1973 and *Pseudomonas* sp (formerly known as *P. viridiflava*) was accepted as the causal bacteria. This pathogen lives as an epiphyte. It mostly affects shaded buds and flowers and its incidence fluctuates between seasons and varied between orchards. Prevalence of this disease was influenced by Spring rainfall. Since the onset of Psa-V,

incidence of bud rot has increased in some orchards and the question is whether this increased incidence is attributable to Psa-V. Whether this “new” budrot is identical to the previously named symptom is open to question and the mix of bacteria causing the complaint remains unclear.

The current trial was undertaken to determine whether Psa sprays, available within the current grower programme, are efficacious against budrot and to try and characterise the bacteria present on the buds. One Psa treatment was the pre-flowering application of the plant growth regulator CPPU (Ambitious®). Since this was the first trial undertaken after this product had been added to the growers spray list, we recorded any plant affects associated with this treatment.

Budrot Symptoms

The recent outbreaks of budrot are characterised by significant leaf spotting in early spring (attributed to Psa) followed by browning of sepals on the hanging buds, browning of petals on the expanding bud and then deformed and brown flowers during flowering. Severely affected buds do not pollinate and may not open. These symptoms are not characterised by a “wet rot” but rather by dry brown flowers and buds with stalks which progressively desiccate to thin cotton-like threads.



Figure 2. Bud browning on buds at flowering. Mildly affected buds appear to open normally while more severely affected buds may only partially open or not open at all.

Trial Design

Design Comments

Such trials walk a tightrope between cost and robustness. They are always at the mercy of seasonal weather and if conditions do not encourage the problem the research money may be spent in vain. In order to minimize this risk the obvious plan is to spend even more – i.e. to replicate the entire experiment on multiple orchards in different regions and with different timings in order to spread the experiment in time and space in order to increase the chances of suitable weather.

This overall experiment was of minimal size comprising 2 orchards: one HW and one G14. Both varieties are of high interest and both had exhibited severe symptoms in previous seasons.

Why look at both varieties? Firstly they may have different propensities to budrot and secondly they flower at different times so including both varieties should increase the chances of appropriate weather encouraging the symptoms at least one site. This report covers only the G14 component.

Approach

The approach was to spray single vine replicates on a G14 orchard with a range of chemicals in the weeks prior to flowering. Both orchards had a history of severe losses to budrot in the previous season.

Experimental Layout

A block which had shown the most severe and widespread budrot damage in previous seasons was used in the trial. With deference to the layout (rows per block, male layout and missing vines) enough vines to cater for all treatments were marked off at one end of the block. The areas included gaps where vines had been removed because of the severity of Psa in the previous season. This experiment targeted female vines whereas the gaps were predominantly in the male rows and consequently did not affect this experiment directly. Treatments were randomised down rows with every treatment in every row (Figure 3). The number of replicates was governed to some extent by the number of rows in the block which we judged to be “uniform”. “Uniformity” in this case was a judgement call since the blocks had some topography and also areas of better and worse vines, perhaps related to the severity of symptoms in previous seasons.

The block was pergola with strip males. Each experimental bay was identified with colour coded ribbons (Figure 4) and the spray treatments were applied by hand using a motorised sprayer (Figure 5). The operator attempted to walk down the row at a constant rate while waving the spray nozzle from side to side to provide a uniform spray coverage across the canopy. Subsequent sampling was focussed towards the centre of each bay to avoid possible interference with adjoining treatments.

		Female row numbers...									
		16	14	12	10	8	6	4	2		
		Rest Of Block									
S H E L T E R	S	3	4	X	X	10	X	7	U	S H E L T E R	
	H	7	1	6	X	X	9	X	U		
	E	10	X	5	9	6	8	10	U		
	L	X	8	X	10	X	1	9	U		
	T	9	X	1	2	3	4	8	U		
	E	5	2	4	4	8	3	2	U		
	R	1	7	9	6	7	6	5	U		
		8	3	8	7	1	2	4	U		
		6	9	10	3	5	5	1	U		
		2	5	7	8	2	7	X	U		
		X	6	3	5	9	10	6	U		
		4	10	2	1	4	X	3	U		
		X	X	X	X	X	X	X	U		
		T		R		A		C		K	

Figure 3. Experimental layout of the trial positioned at the south end of the block. Each square signifies 2 bays with female canopy extending both sides of the leader bounded by (narrow) male rows (odd row numbers, not

shown). Numbers denote treatment codes and X denotes a missing vine, U an unused row. The vertical dashed blue lines indicate the presence of white undervine shelter – see photo below.



Figure 4. 14 Trial block after tagging for treatments.



Figure 5. Spray being applied to a treated bay by motorised knapsack sprayer.

G14 Treatments

The G14 orchard was on the flats to the East of Te Puke on mildly undulating terrain.

The winter copper-based spray programme applied to the block prior to the trial is shown in Table 1. Winter spray programme extracted from the growers spray diary. Another copper spray was to be applied on 4th October but was cancelled on the experimental area because of this trial.

Spray Date	Product	Product Rate g or mL per 100L	Product Rate g or mL per Ha	Justification
15 May 13	Nordox 75 WG	30	300	Psa Mgmt
7 Jun 13	Nordox 75 WG	30	300	Psa Mgmt
24 Jun 13	Nordox 75 WG	30	300	Psa Mgmt
8 Jul 13	Nordox 75 WG	30	300	Psa Mgmt
5 Aug 13	HiCane Driftstop	6% 100	42 L/Ha 700	Budbreak Adjuvant
16 Aug 13	Nordox 75 WG	70	700	Psa Mgmt
3 Sep 13	Nordox 75 WG	50	500	Psa Mgmt

Table 1. Winter spray programme extracted from the growers spray diary.

The experimental treatments are given in Table 2 along with rates and timing of sprays.

Treatment	Trtmt No	Rate	Dates Applied
Control (nothing applied after the trial started)	1		
Kocide Opti	2	70g/100L	7 Oct 2013; 18/Oct/13
Actigard (Foliar)	5	200g/ha	7 Oct 2013; 18/Oct/13
Key Strep	3	60g/100L	7 Oct 2013; 18/Oct/13
Program: Kocide, Actigard Foliar, Strep	4	70g/100L 200g/ha 60g/100L	7 Oct 2013; 18/Oct/13
Kasumin	7	500ml/100L	7 Oct 2013; 18 Oct 13
Ambitious	8	75nl/100L	7 Oct 2013; 18 Oct 13
Actigard (Soil)	6	0.5g/plant	7 Oct 2013; 18 Oct 13
Cane Girdle	9		18 Oct 2013
Trunk Girdle	10		18 Oct 2013

Table 2. Chemicals, rates and timing of sprays applied to the trial

These treatments comprised:

1. Current allowable commercial spray products:
 - a. Kocide Opti®: commercial copper hydroxide formulation
 - b. Actigard®: Foliar applied commercial elicitor consistently found to be the most effective against Psa in greenhouse and potted plant trials.
 - c. Key Strepo: Streptomycin sulphate: clearly effective in trials against Psa, duration of efficacy uncertain.
2. Products licenced as the trial commenced:

- a. Kasumin®: Kasugamycin; newly licenced bactericide effective against Psa in pot trials; expected to be similar to strep although with a different biological mode of action.
 - b. Ambitious®: Commercial CPPU formulation. Plant growth regulator; banned for application post flowering. Few production trials on Psa, side effects unknown but expected.
3. Products or techniques allowed but not of clearly proven efficacy:
- a. Actigard® (soil): should work well; supposedly more systemic than foliar application, application method for entry into plant unknown
 - b. Cane girdle: commonly used 5 weeks after flowering (for size enhancement) or in late Jan/feb for DM improvement. Used in Japan for fighting Psa infection/budrot and showed efficacy in Zespri Italian trial for budrot. Appropriate timing uncertain, perhaps 3 weeks before flowering.
 - c. Trunk girdle: As for cane girdle. Relative efficacy unknown. Is efficacy related to “number of wounds” or level of isolation from the roots?

Assessments

Vines were assessed for Psa symptoms twice after applying treatments.

The first assessment was after applying the treatments (21-25th Oct). We tagged 4 “central” canes per vine and scored each shoot on each cane for:

1. **Extent** of spotting
2. **Severity** of spotting
3. Presence/severity of **budrot**

Scoring was subjective on a 0-5 scale from nil presence of the characteristic through to very severe.

The second assessment was after flowering and was aimed at measuring potential fruit loss. Unfortunately the grower removed all “non-class I” fruit very shortly after the bees were removed from the orchard. This meant we had to modify our 2nd assessment protocol. For this assessment we selected 10 shoots per vine (treatment replicate) on 4 of the marked canes. For each shoot we then recorded

1. number of fruitlets
2. number of brown stalks
3. number of green stalks

The potential fruit numbers were given by the sum of the above. We assumed that the brown stalks were associated with loss due to budrot and the green stalks were from healthy fruit which had been removed for cosmetic reasons. This may underestimate the proportion lost to budrot as fruit which were poorly formed as a result of lightly infected flowers may have been represented by green stalks.

Treatment effects at Harvest

In order to check whether any treatment affected the timing or characteristics at maturity 13 fruit were taken from each vine and tested shortly before harvest for fresh weight, brix, %DM, fruit pressure, and Hue.

Microbiology

Phenotypic characterisation of “bud rot” pathogens

The ICMP cultures (International collection of Microorganism from Plants) listed below were obtained from Landcare Research for comparative study. They were examined using phenotypic and genotypic methods. These strains are associated with, or suspected to be the causal pathogen for budrot and subsequently for fruit loss.

- 1) ICMP 18708 – *Pseudomonas syringae pv actinidiae* (Takikawa et al 1989) isolated in the Te Puke area and characterised as biovar 3 LOPAT 1a
- 2) ICMP 11164-*Pseudomonas syringae van Hall* 1902 isolated in the KatiKati characterised as Lopat 1a
- 3) ICMP 18800 *Pseudomonas syringae pv actinidia* (Takikawa et al 1989) isolated in the Paengaroa region and characterised as biovar 3.
- 4) ICMP 13303 *Pseudomonas viridiflava* (Burkholder 1930) Dowson 1939 Te Puke and characterised by LOPAT II

These cultures were grown on media and a single colony was isolated to study the morphology for comparison of growth in relation to orchard samples collected both pre- and post-treatment application. An oxidase test was conducted.

Genotypic characterisation “bud rot” pathogens

Partial sequencing was carried out on the two strains - ICMPs 13303 and 11164 to establish the differences or similarities between them. Partial sequences of the 16s ribosomal DNA and a partial sequence of the rpo D gene were examined. This was undertaken at the University of Otago by Dr Margaret Butler who collaborated with us on this characterisation of the “bud rot” pathogen.

Copper and Streptomycin Resistance testing of “budrot” causal pathogen and other microflora of kiwifruit vines

The efficacies of copper and streptomycin can be reduced by the occurrence of copper and streptomycin resistant bacteria. A comparative study was conducted to check whether the “bud rot” causal bacteria and Psa: biovar 1 and 3 isolated from Japan, New Zealand and Italy along with other kiwifruit bacteria were resistant to copper and streptomycin.

The agar dilution test is a serial two-fold dilution of the antibiotic in Casitone Yeast Extract Agar. The outcome of this test was to find the lowest concentration of antibiotic that inhibits visible growth on the surface of the agar (MIC). The dilutions of streptomycin were prepared in Casitone Yeast Extract agar to final dilutions of 0 ug/mL, 1 ug/mL, 10 ug/mL and 100 ug/mL. Inoculation on the agar was carried out using multipoint inoculator and plates were incubated for 24-48 hours.

Resistance to copper was determined using a low-complexing mineral salt medium to avoid binding. This was used a modified Casitone Yeast Extract Agar and dilution of copper sulphate was prepared to reach dilutions equal to 0.16 mM, 0.32mM, 0.64 mM and 0.96 mM. Inoculation on the agar was carried out using a multipoint inoculator and plates were incubated for 24-48 hours.

Estimation of epiphytic populations of Psa-V compared to Pseudomonas sp (formerly known as viridiflava)

High populations of particular bacterial species and pathovars are associated with higher probabilities of plant diseases. Epiphytic populations of Psa-V and *Pseudomonas sp* (formerly *viridiflava*) form a significant source of inoculum for spread, and may increase the chances of successful colonisation of adjacent plants and potentially cause “budrot” on buds and flowers. Quantitative comparisons of Psa-V population ICMP 18800 and *Pseudomonas sp* (formerly *viridiflava*) ICMP 13303 under high humidity were carried out on Hayward seedlings.

Due to the hairy nature of the underside of kiwifruit leaves, inoculation of Psa solution onto the underside of leaves was not possible as the droplets would roll off the leaf surface. Based on a preliminary test conducted prior to the experiment, Psa-V and solution and *Pseudomonas sp* were mixed with 0.5% Pulse® to overcome this issue. The inoculum of Psa-V solution and *Pseudomonas sp* used to spike the leaves were 5×10^7 cfu/mL and 2.9×10^7 cfu/mL. Point inoculation was made with 40 μ L of each inoculum on leaf discs. Sampling was carried out at various time interval and culture quantification was conducted to estimate the populations (cfu/mL).

Sample Collection, PCR identification and Culture Quantification

Leaf, bud and cane samples were taken from the orchard site both pre- and post-treatment application to check for the presence and level of Psa-V, *Pseudomonas syringae* and *Pseudomonas sp* (formerly known as *viridiflava*). Both symptomatic and asymptomatic tissues were collected. Samples were double bagged and labelled according to treatment and tissue type. Sampling was carried out aseptically using sterile blades to avoid cross-contamination between samples.

PCR identification

PCR identification was used to screen samples that were positive for Psa-V before culturing. The method used is as outlined:

- a) Plant material was processed (as above) and 1 mL of GPX lysis buffer was added to the material in the tube and bead-beated for 3 minutes.
- b) DNA extractions were made on 500 μ L aliquot of the supernatant collected after spinning and tested by qPCR using a rapid Psa-V test.
- c) qPCR results are given as the replication value (Cq). The lower standard detection limit for Psa-V using our laboratory processes is $Cq \leq 33$.
- d) Identification of Psa-V by qPCR was conducted on all the different types of samples buds, leaf and canes.

Cultures

Some of the samples were parallel streaked on Aitken media, kings B and TSA (Tryptic Soy Agar) plates. Aitken media is a semi-selective media that allows Psa-V to grow with a distinctive morphological characteristic. Colonies are small, roughly circular, smooth and pink in colour. Kings B medium was also used as it is recommended for non-selective isolation, cultivation and pigment production of *Pseudomonas* species. TSA is a general purpose media which supports the growth of a variety of bacteria and fungi. The plates were streaked with a loop of the processed samples tissue and incubated at 25°C for 24-48 hours before observation. For comparison, all reference cultures for bud rot were streaked on each media as positive controls.

Culture Quantification (cfu/mL)

Some of the samples that had high growth of Psa-V on streaking in cultures were further quantified to provide an estimate of Psa-V concentration in cfu/mL. A serial dilution and plating was carried out on Aitken media and incubated at 25°C for about 36-40 hours before reading.

ICMP 18800 Psa-V was used as a reference standard culture for colony comparison of Psa-V. Psa-V colonies are small, roughly circular, smooth and pink in colour. In cases where colonies could not be identified visually, they were checked using qPCR.

Results

Orchard information

Bees were brought onto the orchard for pollination on 30th Oct and removed around the 13th Nov. The area was artificially pollinated using a wet application method at 0.5 kg pollen per Ha on the 4th Nov since the males were generally poor after the Psa losses in the previous season.

This year the crop packed out at ≈8,500 TE/Ha with an average count size of 34. This compares with the estimated production last season from this area of only ≈2,300 TE/Ha attributed to bud loss. Fruit size was disappointing this season as on-vine estimates had pointed to a 32 average count size and supplemental pollen had been applied.

The orchard has weather stations on site. The spring rainfall data are shown in Figure 6.

Observations

At its commencement it seemed there was a good chance that this trial would be over before it started. It is worth including a diary of events.

3 Oct	Thurs	Marked out trial vines and treatments . No spotting observed on any treatment vines
4-6 Oct	Fri-Sun	Fine weather
7 Oct	Mon	Fine weather. Spray treatments applied. Obvious light spotting on some scattered males and sometimes adjacent females. Very variable around the trial area. Nothing like as bad as last season according to manager.
8 Oct	Tue	Leaf samples collected for micro work. Spotting much worse (severity), with some serious blackening and budrot on hanging flowers of both males and some females. Still very variable around the trial area. Overall light/moderate.
9-10 Oct	Wed-Thu	Overcast showery weather
11 Oct	Fri	Browned buds scattered throughout, some blackened and curled leaves in places, spotting common but only a few severely affected areas. Still very variable. The overall worst area of the block was the North-west corner (not in the trial area) where there was a gap in the shelter. There was some (wind) canopy damage in this area

So on the 7th and 8th it appeared we were “too late” to stop a disaster already in progress but by Friday I was puzzled but relieved!

Figure 77 and Figure 88 were taken on the 7th Oct and Figs 9 to 12 on the 8th Oct. Remembering there was no spotting on the 3rd Oct the rapid progression of symptoms *in patches*, including completely browned buds and blackened and cupped leaves, was

remarkable. Given that treatments were applied on 7th and the weather was then wet till Friday I expected to find extensive budrot. To our surprise ***no obvious further increase*** in bud browning was observed for the duration of the trial – including on control vines. If anything symptoms became visually less obvious as the vines continued with normal spring growth. Visually the worst affected areas occurred in rows 8-14.

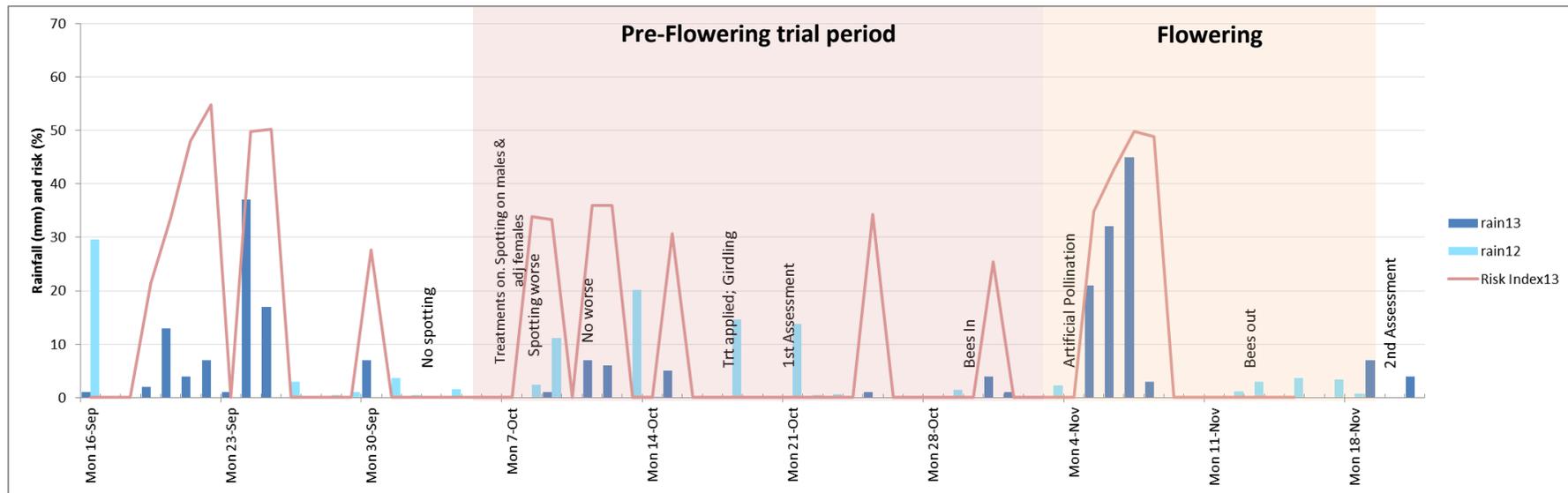


Figure 6. Daily rainfall recorded on the orchard along with an activity diary. Dark blue bars are 2013 rainfall, light blue 2012 and the red line is the KVH risk index for 2013.



Figure 7. 7th Oct Leaf spotting.



Figure 8. 7th Oct Leaf spotting



Figure 9. 8th Oct. Widespread and more intense leaf spotting than 7th Oct in some areas.



Figure 10. 8th Oct Some leaves had gone from spots to “blackening”. Such leaves are cupped (“scrunched”) upwards in a similar manner to the “3 cornered hat” look of severely water-stressed leaves. These symptoms have been seen previously on HW in areas badly affected by Psa. The upper surface of the leaf is shown in the next figure.



Figure 11. 8th Oct. Opening out a cupped and blackened leaf. It appears the rapid death of a large area of central tissue pulls the leaves into the characteristic “scrunched” look.



Figure 12. 8th Oct. Early light browning of sepals on hanging buds



Figure 13. 9th Oct. Various levels of browning on a cluster of buds



Figure 14. Badly affected browned buds on the 11th Oct. Some buds and stalks completely brown



Figure 15. 11 Oct. Wind damage and budrot in the NW corner of the block (not in the trial area). Notice the spotting and browned buds.

Ambitious (CPPU) visual effects

After spraying Ambitious (CPPU) we made observations of any unusual plant growth affects associated with this treatment. All these effects were also observed on the companion HW trial and some (identified) photographs included here are from that trial.

Red leaves

Within a few days of spraying the currently growing shoot tips and leaves took on a red colour (Figure 16 and Figure 17). This persisted for some weeks before gradually becoming less prominent.

Late buds

Ambitious® seemed to foster the development of late and small buds which we would not normally expect to develop. This had the effect of slightly prolonging flowering as late, often small, buds continued to open (Figure 18 and Figure 19). These flowers varied from being “normal”, but a little late, through to poorly developed or deformed buds with minimal or no flower parts.



Figure 16. 18th Oct. Growing tips became red after treatment with Ambitious® (CPPU).



Figure 17. 18th Oct. Red colouration on actively growing shoots treated with Ambitious®.



Figure 18. 18th Oct. Extremely late partially developed buds which attempted to flower after being sprayed with Ambitious®. Normally developing fruitlets are also shown.



Figure 19. 18th Oct. Extremely late and small flowers opening after being treated with Ambitious®. No petiole.

Fat stalks and buds and thicker leaves

Within a short time of spraying it became apparent that at least some stalks of treated fruit became fat and often redder (Figure 20 to Figure 22). The buds also appeared larger however when the flowers opened we looked at both the size and thickness of the petals Figure 23. The individual petals were considerably larger on the Ambitious treated vines and

were also considerably thicker (heavier per unit area). Increased thickness of leaves on treated vines was also observed on both HW and G14. This did not apply to all leaves – it appeared that leaves which were growing at the time of application were affected but leaves which developed subsequently were not.



Figure 20. 17th Oct Fat red stalks on buds (with browning) treated with Ambitious®.



Figure 21. 31st Oct. HW buds showing the fat red stalks associated with Ambitious® treated vines.



Figure 22. 31st Oct. Ambitious® treated HW buds compared with a non-treated bud (held). Note the bud size, stalk size and colour differences.



Figure 23. A HW flower from a control vine (left) compared with a single leaf petal from an Ambitious® treated vine.

Hairy (gingery) fruit

A minority of fruit sprayed with Ambitious® stood out for their level of “hairiness” and the fact that they had a gingery colour (Figure 24). Some of these fruit also had a slight more pointed distal end.



Figure 24. HW fruit photographed on 20th Jan 2014 comparing a normal fruit (left) with an extreme example of the “ginga” syndrome brought about by Ambitious® spraying pre-flowering (right).

Vine Scoring Results

All scoring was undertaken by one person. While scoring is subjective it was hoped the advantage of a single viewpoint would outweigh the disadvantage of the additional time it would take one person to score the trial.

First Round: post treatment application

Individual scores (0-5) were given for

1. **Extent** of spotting (how widespread it was)
2. **Severity** of spotting (i.e. small spots? Complete leaf blackening?)
3. Level of **bud browning** on the hanging buds

Two different scoring approaches were used: either every shoot on 4 canes per vine was scored individually, or each of 4 canes was given a score. The change was made because it was taking too long to complete the evaluation using the more detailed and slower method.

Figure 25 shows a treemap for the Extent of leaf spotting. A treemap (by row in this case) is a useful means of visualising both the level and variability of a statistic. Each rectangle represents a rep (vine): its colour is assigned by treatment and its area corresponds to the level of the statistic.

Clearly row 4, on the eastern side of the block, had a much higher **extent** of spotting than any other row. Spotting was least on the west (row 16). The inner rows were similar to each other with slightly higher spotting extent towards the middle of the block. Treatment efficacy can be evaluated by looking both along rows and vertically (between rows). There appears to be a relatively narrow range of efficacy between treatments: ActiS is generally of high **Extent** (i.e. it was ineffective in reducing spotting) whereas cane girdling and Strep had low Extent (prevented spotting). Generally none of the treatments stand out i.e. when averaged across rows none of the treatments looks to be dramatically different from the others.

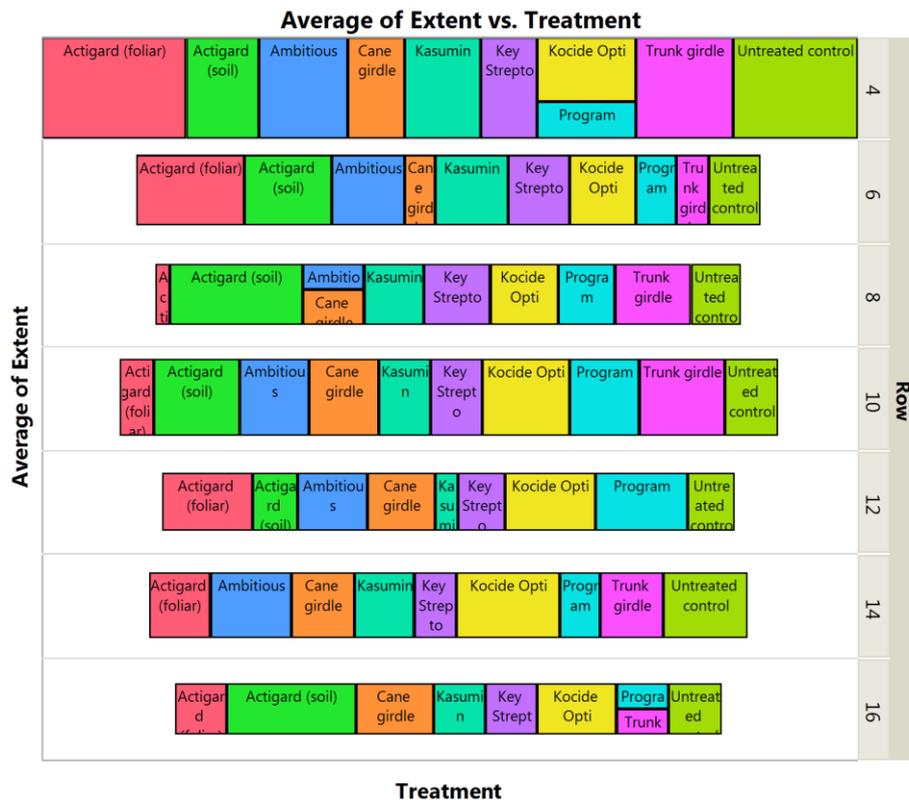


Figure 25. Treemap of the *Extent* leaf spotting of G14 leaves following *Psa* treatments prior to flowering. Treatments are shown in alphabetical order within rows, not geographical order.

ANOVA results for *Extent* are shown in Figure 26 and Figure 27, and Table 3 and Table 4. They show there is no significant difference by treatment but there is by row as we might expect from our observations above.

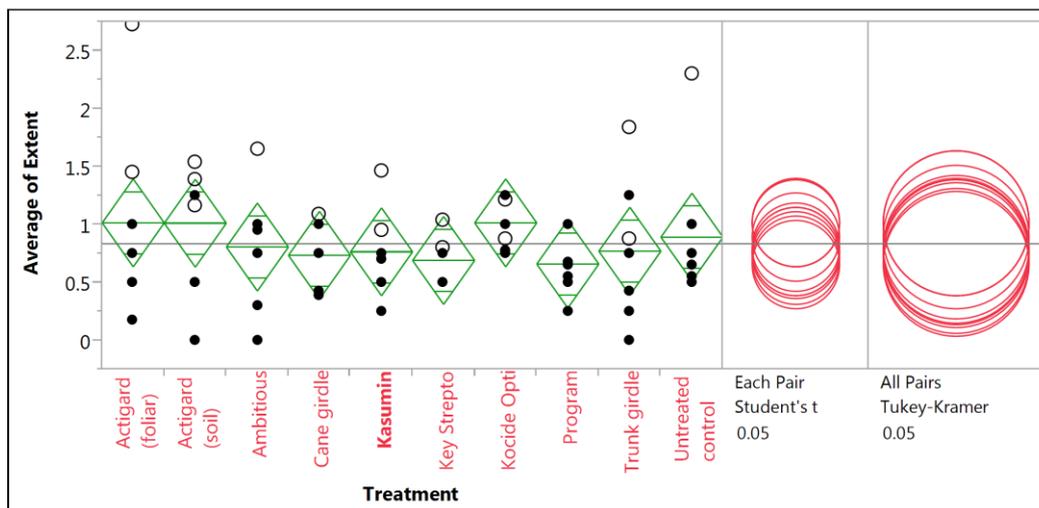


Figure 26. Analysis of variance for *Extent* vs treatment showing spread of data and means testing circles at the right.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	9	1.196823	0.132980	0.5281	0.8484
Error	60	15.107686	0.251795		
C. Total	69	16.304509			

Table 3. ANOVA for *treatments* showing no significant difference overall.

Treatment	Extent	Treatment	Severity	Treatment	Bud browning
Actigard (foliar)	A 1.0157	Kocide Opti	A 1.0871	Kocide Opti	A 0.9971
Kocide Opti	A 1.0157	Program	A 1.0229	Actigard (soil)	A B 0.8471
Actigard (soil)	A 1.0128	Actigard (soil)	A 0.9543	Key Strepto	A B 0.8329
Untreated control	A 0.8928	Actigard (foliar)	A 0.9414	Program	A B 0.7471
Ambitious	A 0.8072	Cane girdle	A 0.9129	Cane girdle	A B 0.7057
Trunk girdle	A 0.7714	Untreated control	A 0.8771	Ambitious	A B 0.6857
Kasumin	A 0.7657	Ambitious	A 0.8286	Kasumin	A B 0.6014
Cane girdle	A 0.7357	Trunk girdle	A 0.7757	Actigard (foliar)	A B 0.5857
Key Strepto	A 0.6914	Kasumin	A 0.7557	Untreated control	A B 0.5729
Program	A 0.6600	Key Strepto	A 0.7171	Trunk girdle	B 0.5214

Table 4. Treatment means ordered from highest (least effective) to lowest (most effective) treatment for Extent, Severity and Bud browning.

If we look at the ANOVA by row (rather than by treatment, Figure 27) we see that row 4 is significantly higher (as we noted above) and row 16 is significantly lower (Table 6) – as we expected from the treemap above.

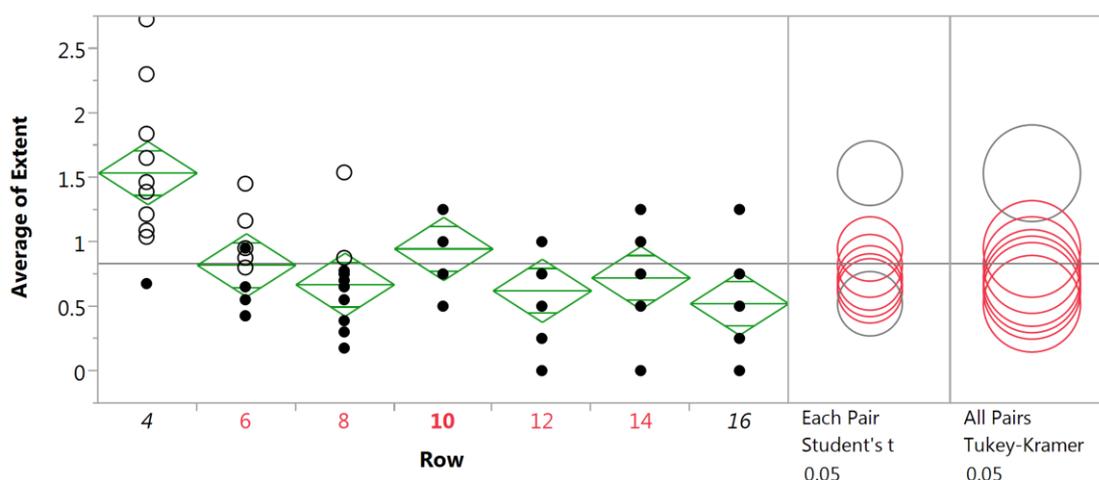


Figure 27. Analysis of variance for Extent vs row number showing spread of data and means testing circles at the right.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Row	6	6.864229	1.14404	7.6348	<.0001*
Error	63	9.440280	0.14985		
C. Total	69	16.304509			

Table 5. ANOVA for rows showing significant differences.

Bow number				Mean Extent
4	A			1.538
10		B		0.950
6		B	C	0.823
14		B	C	0.725
8		B	C	0.672
12		B	C	0.625
16			C	0.525

Table 6. Row means ordered from highest (least effective treatment) to lowest (most effective treatment) along with connecting letters (Students t).

The corresponding treemaps for **Severity** of leaf spotting and **bud browning** are shown in Figure 28 and Figure 29.

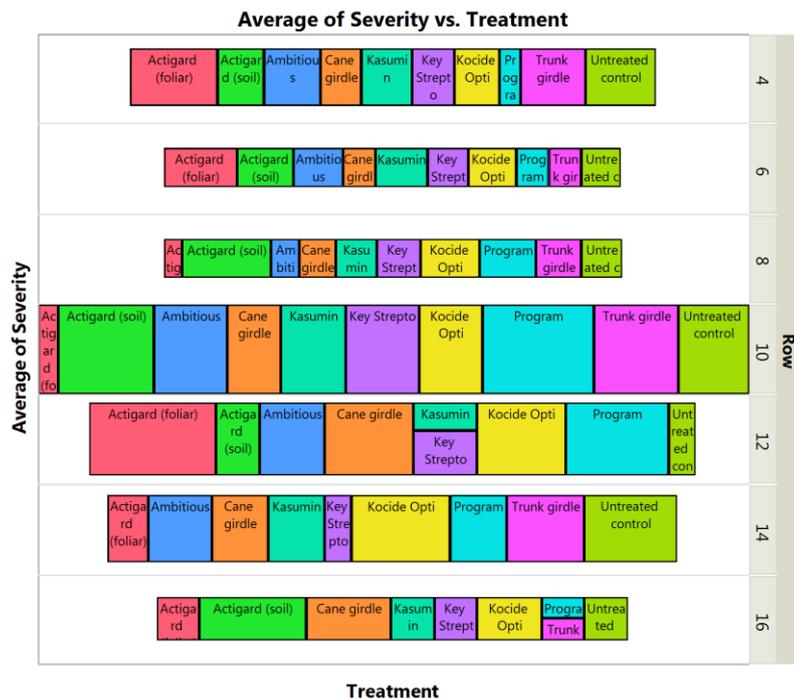


Figure 28. Treemap of the Severity leaf spotting of G14 leaves following Psa treatments prior to flowering.

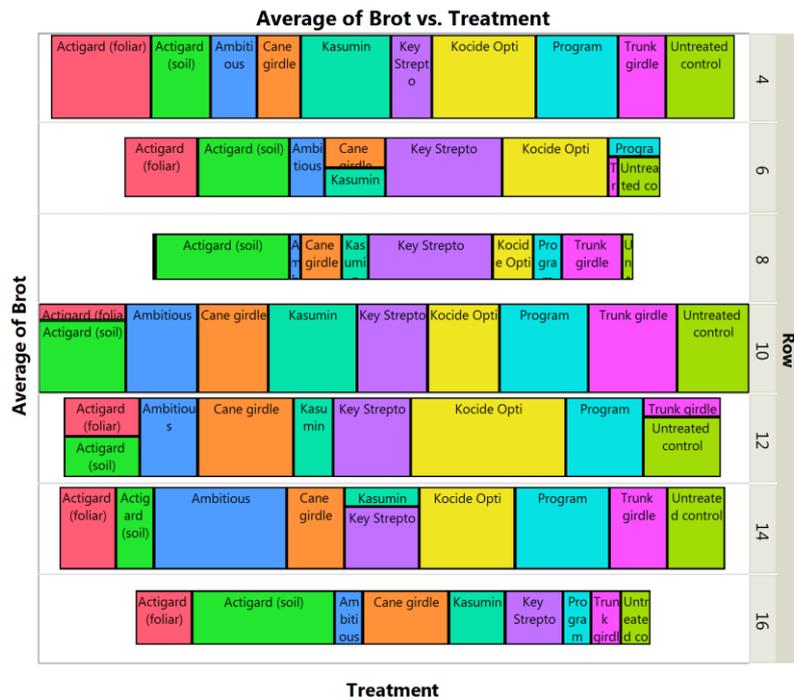


Figure 29. Treemap of the level of Bud Browning following Psa treatments prior to flowering.

The map for **Severity** is quite different from the map for **Extent**. It shows greatest **Severity** in row 10 with very low **Severity** in rows 16, and 4-6. Again, looking across the entire block no one **treatment** seems very different from the others as regards **Severity** of spotting.

Treatment averages for all statistics were given in Table 4 (above). **Severity**, like **Extent** showed no treatment differences while **Bud Browning** showed rows 4 and 16 to be higher and lower (respectively) than the average. We note that the order of efficacy in Table 4 varies for each characteristic scored and they do not generally correspond with the

“expected” order of efficacy that we might expect from other Psa spray efficacy trials. Figure 30 and Figure 31 show this in graphical form.

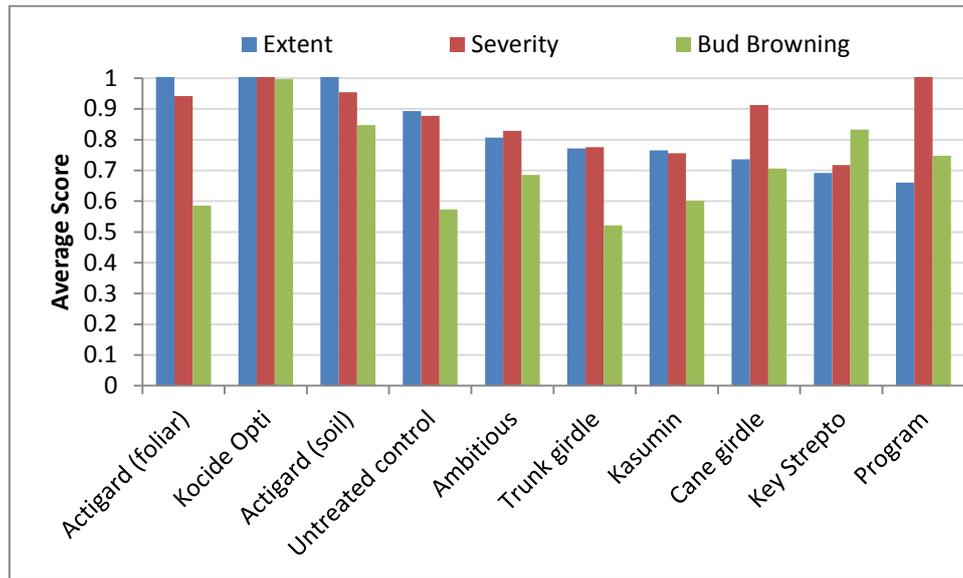


Figure 30. Mean scores for pre flowering assessments of Extent and Severity of leaf spotting and Bud Browning by treatment.

Given the lack of readily identifiable trends in Figure 30 we have plotted the **rank**s of each statistic by treatment in Figure 31. Note that rank ordering will tell us nothing about *relative magnitudes* of efficacies. High values for each statistic correspond with **poor** outcomes so a low rank corresponds to a better treatment. We see that the data falls in to 3 groups:

1. The first 3 treatments: all statistics in the top rankings i.e. poor treatment efficacy
2. Next 4 treatments: all stats in lower ranking = better efficacy
3. Last 3: rankings all over the place

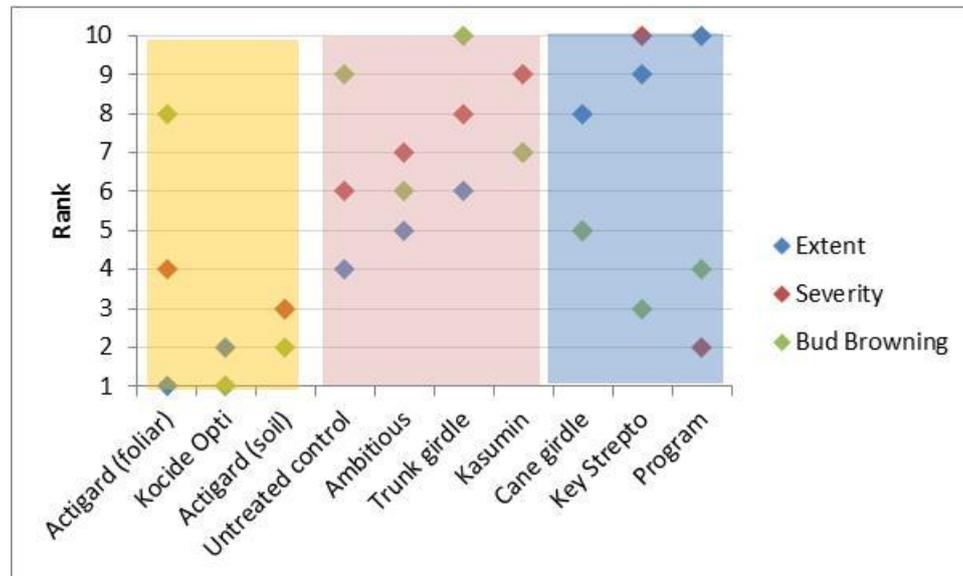


Figure 31. Ranks of Extent, Severity and bud browning by treatment. The first group (coloured bands) is generally good i.e. top half of rankings; the second poor i.e. bottom half of rankings and the 3rd a mixture of rankings. This figure tells us nothing about *relative efficacy*. The treatment order on the x axis is that for Extent which explains the linear increase in that data in the figure.

As stated above, these groups do not correspond with our current expectations as to the efficacy of these treatments for Psa control.

Let's look a little harder at the relationships between **Severity** and **Extent** of spotting and levels of **bud browning**. Figure 32 shows scattergraphs of these variates.

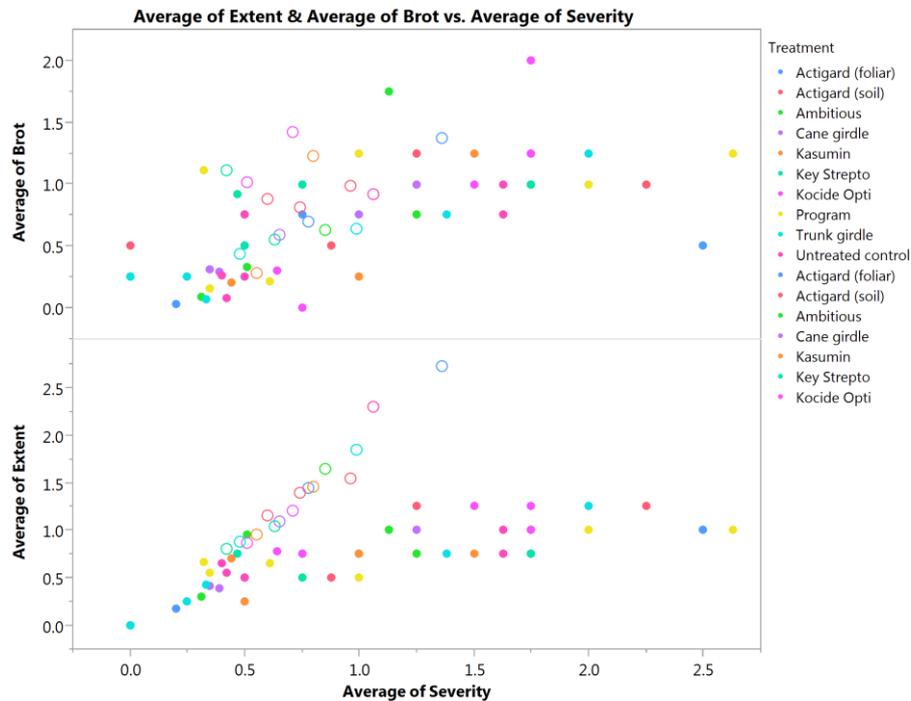


Figure 32. Scattergraphs of Extent of leaf spotting and level of bud browning vs Severity of leaf spotting coloured by treatment. The data showing a linear relationship between Extent and Severity was selected on this graph and the symbol changed to circles.

1. The relationship between **Severity** and **budrot** is one that “tops out” – **bud browning** increases with severity score up until ≈ 1.25 after which there is no further increase in **bud browning**.
 - a. There was no obvious relationship with treatment (colour of dot).
2. The data in the lower graph bifurcated.
 - a. The lower arm followed the same trend as the budrot relationship i.e. Extent didn't increase with Severity after Severity reached around 1.25.
 - b. The upper linear arm of the graph shows some vines for which the Extent continued to increase (linearly) with Severity. Again this relationship was not influenced by treatment.
 - c. We have changed the plotting symbol in both graphs (to a circle) to allow the vines which had this linear relationship to be identified in other graphs. We didn't change the symbol for the low values of Severity where data could be placed in either category.
 - d. The circles are not prominent in the top pane (i.e. in relation to bud browning).
3. We can look for a row relationship by plotting treatment by row (Figure 33) Treatment order is alphabetical not geographical).

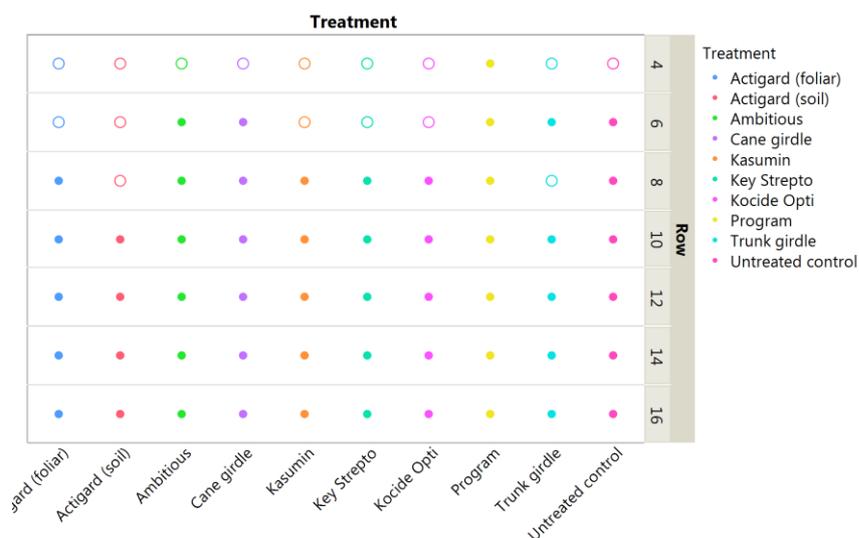


Figure 33. Treatments by row using symbols from Figure 32. The circles are those where Severity of spotting increased with Extent of spotting.

Again the circles identify the upper arm of the previous relationship and clearly the “odd” two rows were the easterly 2 rows. In these rows the **Extent** and **Severity** of spotting are unrelated to treatment but where there was more spotting it was also more severe.

From looking at **Extent** and **Severity** we might expect row 10 to be the most **at risk** of bud browning if we postulate that the risk is related to the *product* of **Extent** and **Severity** i.e. there should be more chance of **bud browning** where leaf symptoms are both extensive and severe.

Second Round Scoring

The second round of scoring was planned to assess fruit loss post pollination. Since the pollinated flowers were removed before this assessment could take place we relied on the colour of the remaining intact fruit stalks to assign losses to either cosmetic thinning (green stalks) or budrot (brown stalks). While this approach was the only one available to us it may minimise the assessed budrot loss if minor budrot had caused deformed fruit with healthy stems. By counting hanging fruit, brown stems and green stems we can assess the total loss and attribute that to either budrot or cosmetic causes.

The data was very variable which we attribute to the variable levels of browning seen around the orchard immediately after the treatments were applied. On average we found 14% loss to budrot and 8% cosmetic loss leaving 78% of the flowers retained as fruit. Figure 34 shows the variation in these values across the block by row.

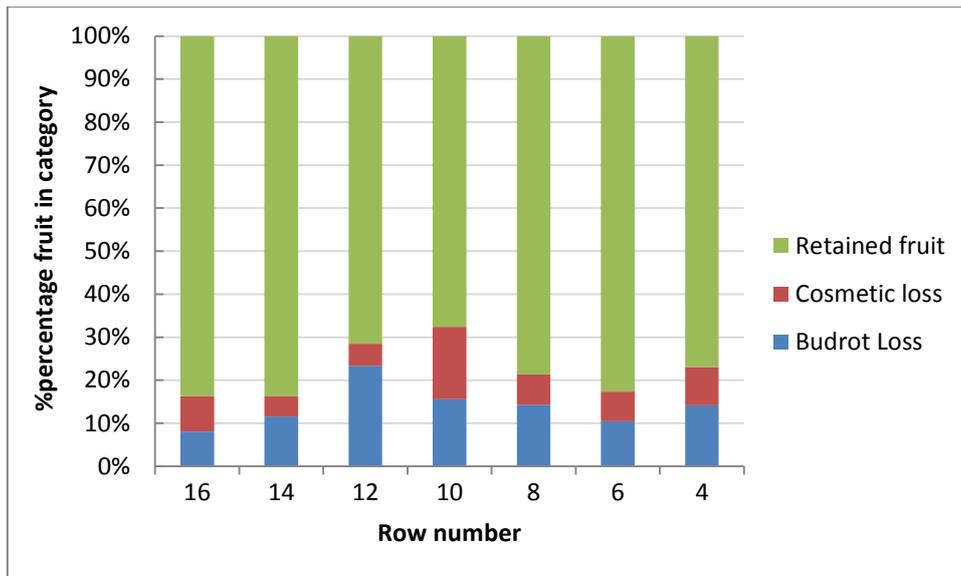


Figure 34. Variation in fruit losses across the block by row. Row numbers are shown reversed in the “geographical” position.

The higher budrot losses were found in rows 8-12.

Again a treemap provides a useful visualisation of the data (Figure 35). Low treatment efficacy is indicated by high budrot losses which are visualised by large rectangular areas in this visualisation.

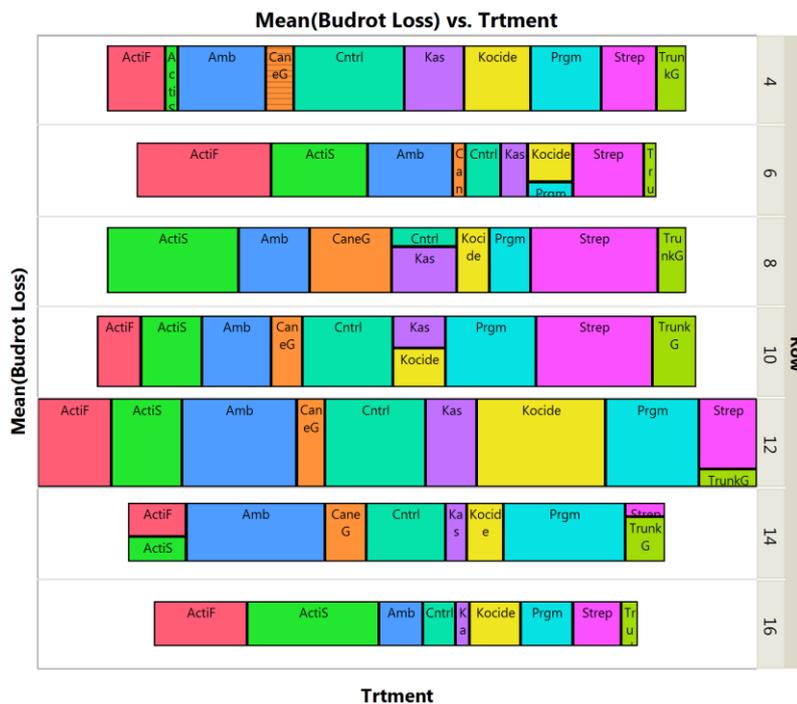


Figure 35. Treemap of budrot loss of G14 fruitlets assessed post-flowering.

Again rectangle colour corresponds with treatment and the area of each rectangle is an indication of the budrot loss so, for example, it is easily seen that all losses (independent of treatment) were low in rows 16, 14 and 6 (smallest overall area) whereas row 12 had significantly more losses than any other row. ActiS was *relatively* ineffective in row 16 compared with row 12, even though overall levels were higher in row 12.

The variability between rows for individual treatments is shown by comparing a colour (treatment) vertically from top to bottom e.g. strep (pink) had high losses in in rows 8 and 10 and smaller losses in rows 12, 16, 14 and 4. Trunk Girdling (yellow-green) was associated with low losses in all rows whereas cane girdling (orange) had one outlier (relatively high in row 8) and had very low losses in all the remaining rows.

Turning to statistics Figure 36 shows the average losses by treatment ordered from worst (left hand side) to best. The cosmetic loss was relatively constant by treatment (average 8%; standard deviation 1.7; red bars) whereas bud browning losses varied from 5.7% to 21.3% (average 14%; standard deviation 5; blue bars) with treatment applied.

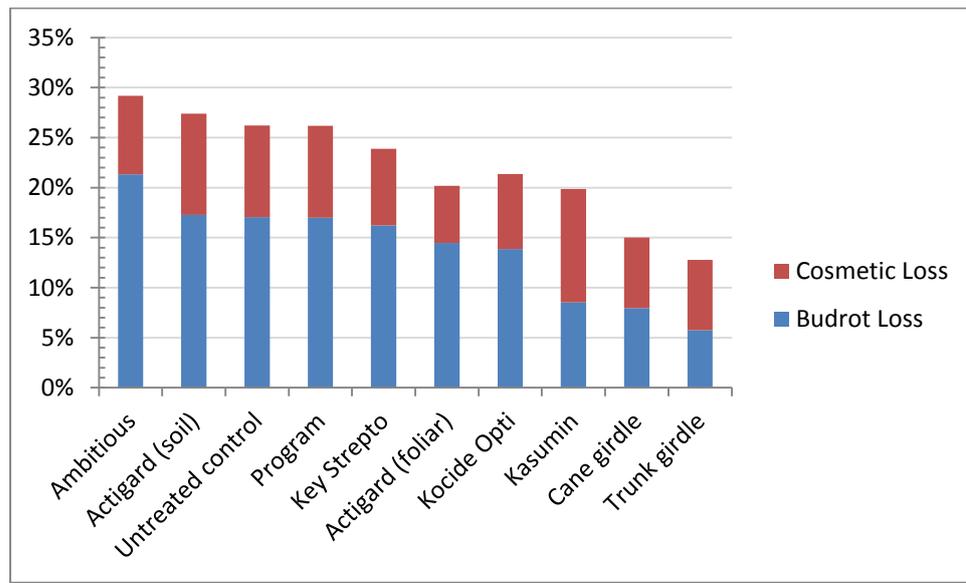


Figure 36. Cosmetic and budrot losses by ordered by treatment efficacy (best on the right).

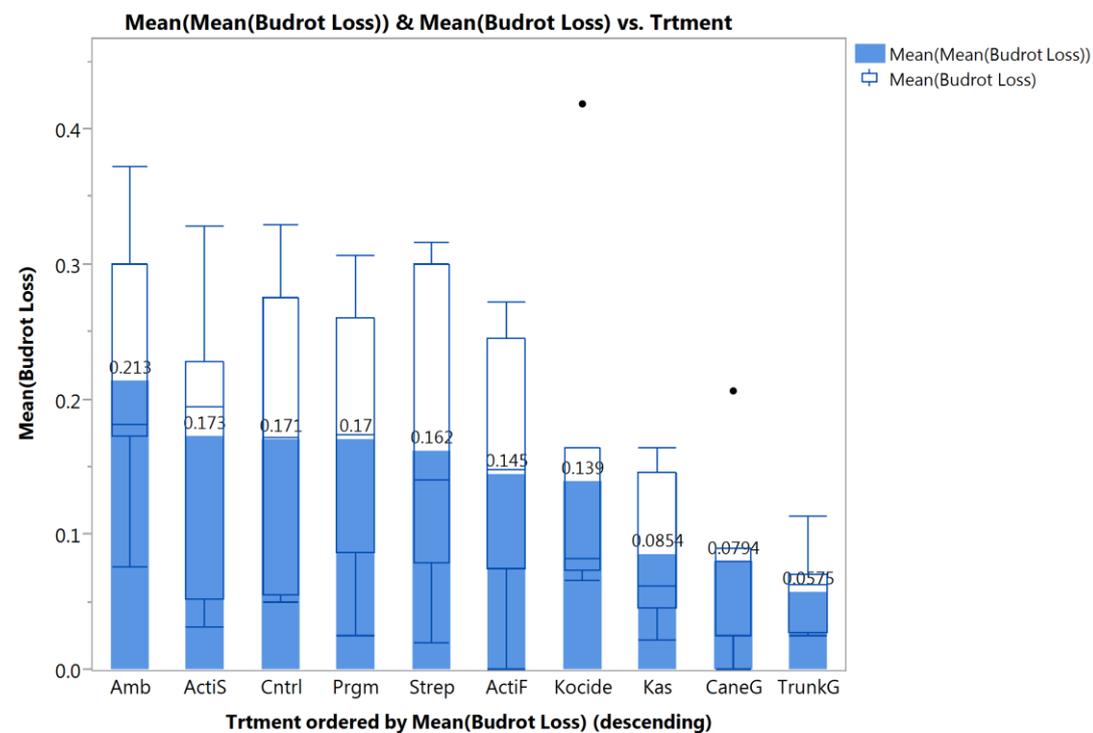


Figure 37. Mean budrot losses ordered from worst to best (left to right) with box plots showing the spread of data, statistical outliers as black points, and mean losses specified.



We can look for statistical differences between treatments using Students t or Tukey-Kramer HSD tests from an analysis of variance. (Figure 38 and Table 7).

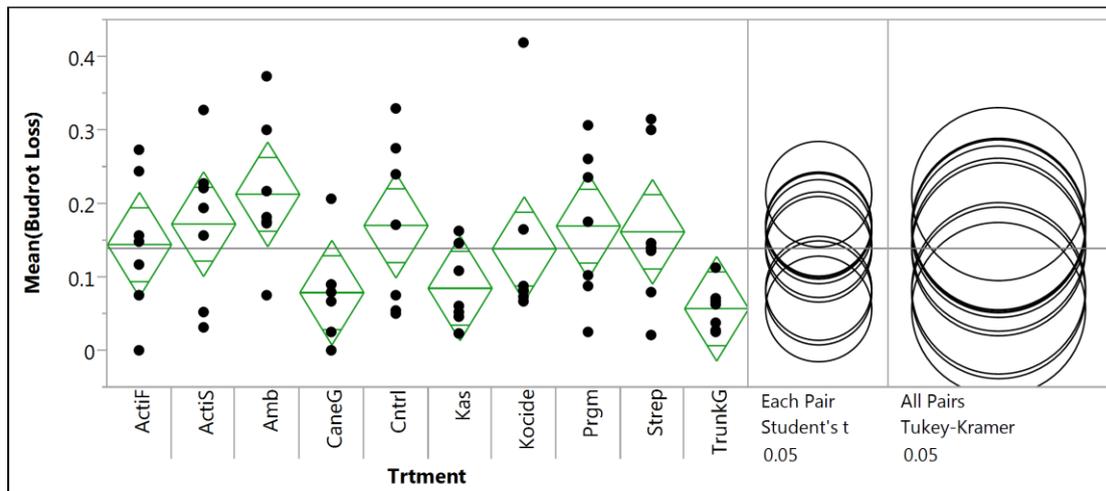


Figure 38. ANOVA of % budrot loss for each treatment in alphabetical order. Students t and Tukey-Kramer HSD circles are shown on the right, details below.

Students t	Budrot loss				Cosmetic loss	
Treatment	mean				mean	
Amb	A			21.3 %	A	7.8 %
ActiS	A	B		17.3 %	A	10.1 %
Cntrl	A	B		17.1 %	A	9.1 %
Prgm	A	B		17.0 %	A	9.2 %
Strep	A	B		16.2 %	A	7.7 %
ActiF	A	B	C	14.5 %	A	5.7 %
Kocide	A	B	C	13.9 %	A	7.5 %
Kas		B	C	8.5 %	A	11.3 %
CaneG		B	C	7.9 %	A	7.1 %
TrunkG			C	5.7 %	A	7.0 %

Table 7. Connecting letters reports for budrot loss and cosmetic fruit loss comparing all pairs using Students t, alpha=0.05. Levels not connected by same letter are significantly different. Treatments in alphabetical order.

Table 7 includes the connecting letters report showing the bulk of treatments were similar (B's). Ambitious® was less effective (the A grouping) and Trunk girdling was more effective (the C grouping). With multiple comparisons the effective level of rejection is a little more generous than the nominal set value (0.05 here). The Tukey-Kramer HSD test keeps more stringently to the designated alpha. Using this test there is **no significant difference** between any of the treatments at this 2nd assessment as shown by the circles in Figure 38.

Position on Canes

For rows 4 to 8 where each shoot was monitored we can look to see if symptoms were located preferentially near the leader or at the end of canes adjacent to male vines. Figure 39 shows the effect for each of the 3 measured characteristics averaged over these rows. It appears that **Extent** increased most strongly on almost all treatments towards the end of canes adjacent to male vines. By contrast **bud browning** showed a much smaller tendency to increase towards the ends of canes.

In relation to *treatment*, Program, Cane girdle and Strep show the least tendency to increase at the outer end of the canes.

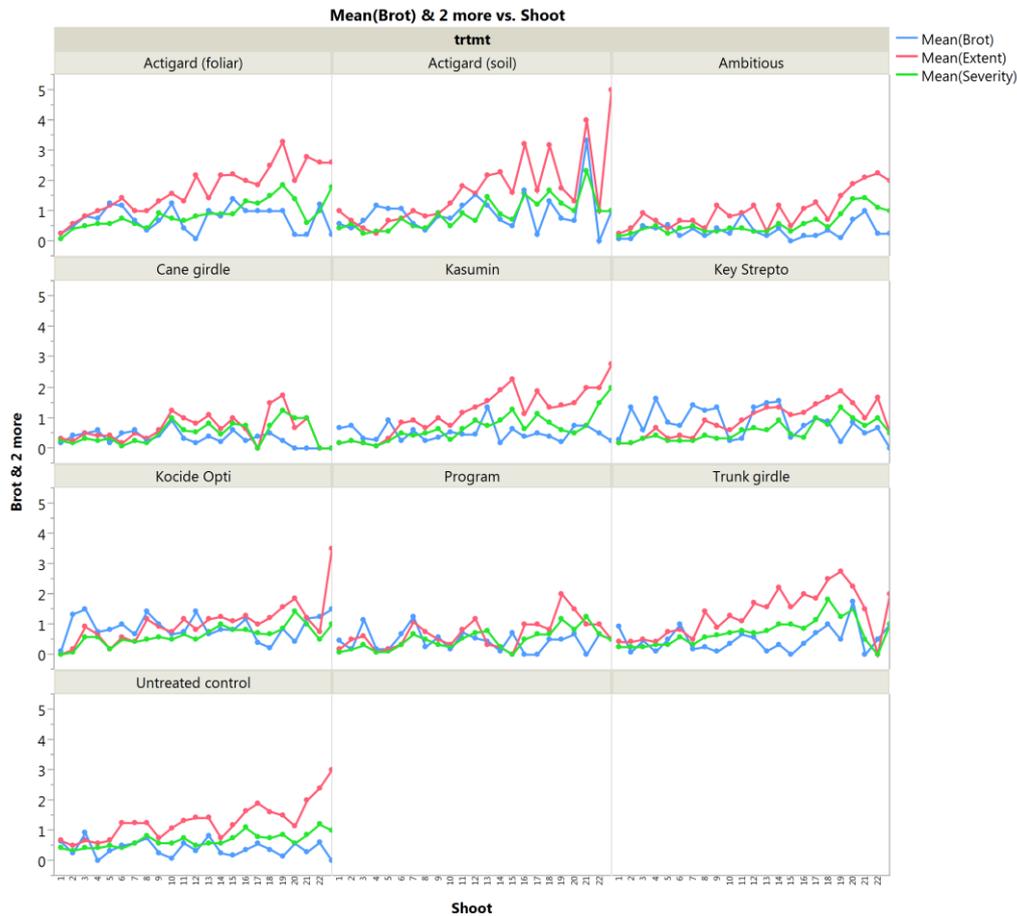


Figure 39. Mean Extent, Severity and Budrot down canes on rows 4-8. Measurements were made on each shoot starting at the leader.

Treatment Effects at Harvest

Figure 40 shows the fruit pre-harvest characteristics by treatment on the 25 Mar 2014.

The control vines appear to show the most difference from the rest however, the measured differences don't point to a simple trend in either fruit quality or maturity. They have a lower hue but a higher pressure and DM and nothing very different in brix whereas for greater maturity we might expect lower pressure, lower Hue and higher brix. The other treatments show odd differences in some characteristics but no consistent trends.

Hue and Pressure showed statistically significant differences between treatments whereas there were no statistical differences in DM, Fresh weight or Brix with treatment.

We also looked at effects by row, DM was the only variate which showed any statistically significant differences across the block – it increased smoothly from East to West (Figure 41).

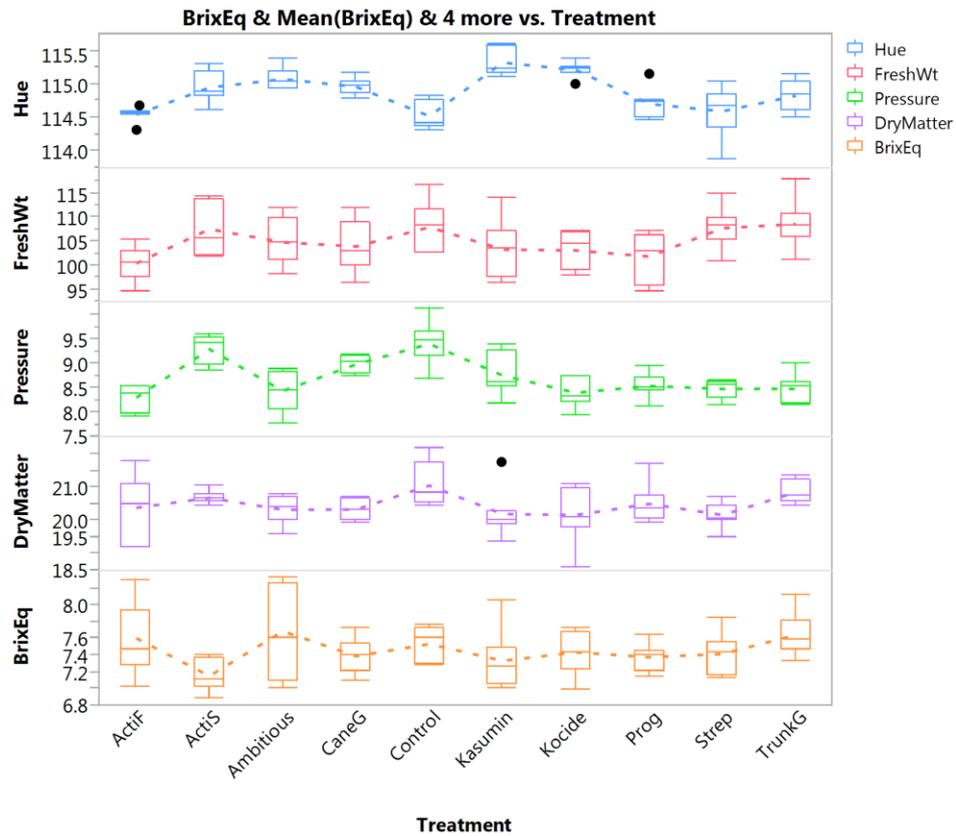


Figure 40. Fruit characteristics by treatment prior to harvest. The dashed line simply joins the treatment means to highlight differences.

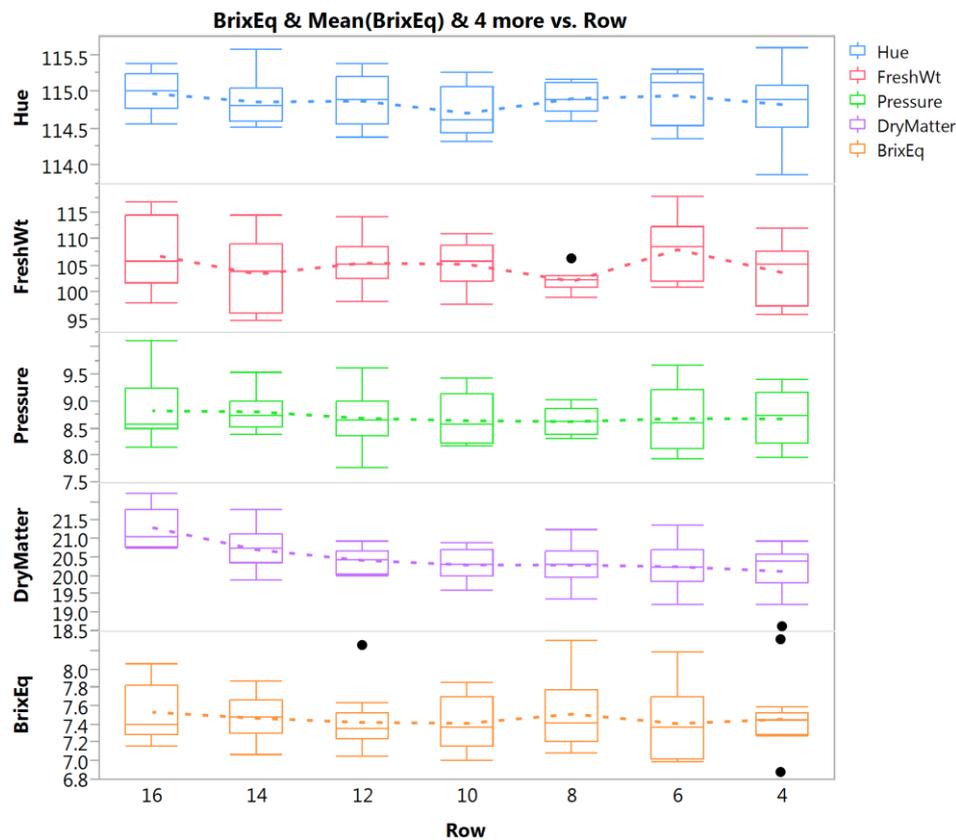


Figure 41. Shows the trends across the block by row. Only DM showed a consistent and statistically significant trend across the block.



Microbiology

Phenotypic characterisation of “bud rot” pathogens

The 4 ICMP cultures were used as positive controls for comparison with species from orchard samples.

ICMP 18708 – *Pseudomonas syringae* *pv actinidiae* (Takikawa et al 1989) isolated in the Te Puke area and characterised as biovar 3 LOPAT 1a and ICMP 18800 *Pseudomonas syringae* *pv actinidia* (Takikawa et al 1989) isolated in the Paengaroa region and characterised as biovar 3 were plated on media H. Psa-V colonies are small, circular, smooth and pink in colour on this media.



Figure 42. Typical colonies of Psa-V ICMP 18708 on Aitken media

ICMP 13303 *Pseudomonas viridiflava* (Burkholder 1930) Dowson 1939 Te Puke and characterised by LOPAT II were used cultured on kings B and Aitken media. Colonies are smooth, circular and creamish in colour with fluorescent pigment production on Kings B media. On Aitken media, these colonies had a different morphology from Psa-V which made them easily distinguishable. Colonies were medium size, smooth, circular, flat colonies, dark grey and produced black pigmentation.



Figure 43. Typical colonies of *Pseudomonas* sp (formerly called *viridiflava*) ICMP 13303 on Kings B media.

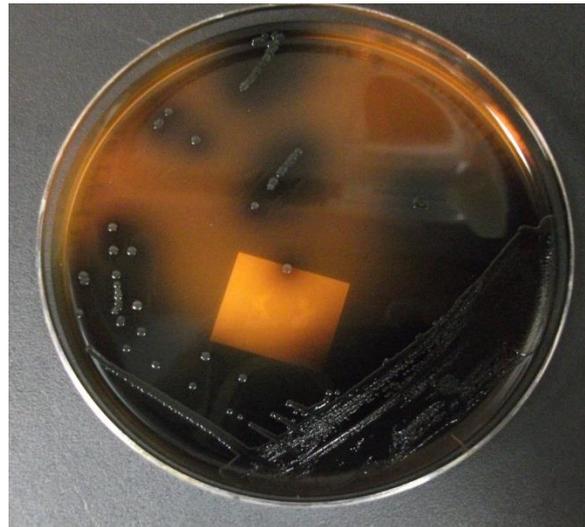


Figure 44. Typical colonies of *Pseudomonas* sp (formerly called *viridiflava*) ICMP 13303 on Kings B media.

ICMP 11164-*Pseudomonas syringae* van Hall 1902 isolated in the KatiKati characterised as Lopat 1a was cultured on kings B media. The colonies were small, circular and smooth in shape on kings B agar. There was no pigmentation or fluorescence as compared with the *Pseudomonas* sp (formerly called *viridiflava*).

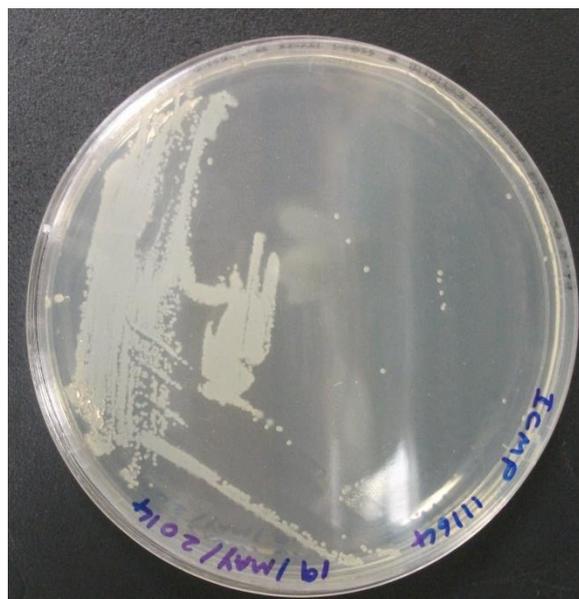


Figure 45. Typical colonies of *Pseudomonas syringae* van Hall 1902 on Kings B media

Genotypic characterisation “bud rot” pathogens

Partial sequencing of the two strains - ICMP 13303 and 11164 seemed to raise more questions than it answered. The *Pseudomonas* sp (formerly called *viridiflava*) strain seemed to have sequences more like some *P. syringae* pathovars but matched no sequence in Genbank. Similarly the *P. syringae* van hall seemed more akin to *P. syringae* pv *tomato* or other close relatives. The “bud rot” or “blossom blight” pseudomonads common on kiwifruit in New Zealand are not similar to *P. viridiflava* in the strict taxonomic sense. *P. viridiflava* has been used historically for these species based on the LOPAT II identification scheme which is

a determinative scheme for fluorescent plant pathogenic pseudomonads (L= Levan, O= Oxidate, P=Pectolytic, A=Arginine, T=Tobacco).

Copper and Streptomycin resistance testing of “bud rot” causal pathogen and other microflora of kiwifruit.

Strains of plant pathogenic *Pseudomonas syringae* and some non-pathogenic bacteria isolated from kiwifruit vine material from local and other countries were compared in the copper and streptomycin resistance testing. Out of the 18 cultures tested, 2 *Pseudomonas* strains - *Pseudomonas sp (viridiflava)* and *Pseudomonas fluorescens* were found to be resistant to copper at the threshold of 0.32 mM and slightly resistant to streptomycin at 1ug/mL.

The *Pseudomonas syringae van Hall 1902* and Psa-V from New Zealand, Japan and Italy was found to be non-resistance to either copper or streptomycin.

Estimation of epiphytic populations of Psa-V compared to Pseudomonas sp (formerly known as viridiflava)

This trial was conducted in a high humidity greenhouse. Both Psa-V and *Pseudomonas sp* (formerly *viridiflava*) survived on the lower surface of leaves for the entirety of the trial despite **no** spotting, or symptomatic leaf tissues, being observed.

Following an initial recovery of Psa-V at 2.3×10^5 CfU/mL, 2 hours post inoculation; the population peaked at 4.5×10^7 cfu/mL at 5 days after inoculation before dropping to 6.2×10^6 CfU/mL at the end of the 7 days trial. Results for *Pseudomonas sp* (formerly *viridiflava*) followed the same trend as Psa-V with a slightly higher initial recovery of 2.6×10^6 cfu/mL at 2 hours post- inoculation. The population of this species peaked after 48 hours to give a recovery of 1.56×10^8 cfu/mL which had only reduced a little by 11 days post inoculation.

Pseudomonas sp population trends showed rapid growth in the first 48 hours of inoculation compared to Psa-V which took 5 days to peak. Since, the trial for Psa-V survival ended after 7 days, it is not possible to comment on when the population trend plateaus or dips as compared to that of *Pseudomonas sp*.

Culture	Lab ID	Source	Area isolated	Disease	Host	Copper Cu (mM)					Streptomycin (µg/mL)		
						0	0.16	0.32	0.64	0.96	1	10	100
<i>P s morsprunorum</i> (Control: resistant strain)	SF 7/3-14	Plant & Food	Not known	bacterial canker	Cherry	G	G	G	NG	NG	G	G	NG
<i>Pseudomonas viridiflava</i> ¹	ICMP 372	Landcare	Auckland	Blossom blight	Kiwifruit	G	G	G	NG	NG	G	NG	NG
<i>Pseudomonas viridiflava</i>	ICMP 13303	Landcare	Te Puke	Blossom blight	Kiwifruit	G	G	G	Ng	NG	G	NG	NG
<i>Psa-V</i>	ICMP 18800	Landcare	Paengaroa	bacterial canker	Kiwifruit	G	NG	NG	NG	NG	NG	NG	NG
<i>Psa-V</i>	ICMP 18708	Landcare	Te Puke	bacterial canker	Kiwifruit	G	NG	NG	NG	NG	NG	NG	NG
<i>Psa-V</i>	ICMP 9853	Landcare	Japan	bacterial canker	Kiwifruit	G	NG	NG	NG	NG	NG	NG	NG
<i>Psa-LV</i>	ICMP 18883	Landcare	Nelson	leaf necrosis	Kiwifruit	G	NG	NG	NG	NG	NG	NG	NG
<i>Psa-LV</i>	ICMP 18806	Landcare	Pongakawa	leaf necrosis	Kiwifruit	G	NG	NG	NG	NG	NG	NG	NG
<i>P s halo actinidiae</i>	Unknown 1	VLS	Te Puke	kiwifruit microflora	Kiwifruit	G	NG	NG	NG	NG	NG	NG	NG
<i>P s halo actinidiae</i>	Unknown 2	VLS	Te Puke	kiwifruit microflora	Kiwifruit	G	NG	NG	NG	NG	NG	NG	NG
<i>P s halo actinidiae</i>	Unknown 3	VLS	Te Puke	kiwifruit microflora	Kiwifruit	G	NG	NG	NG	NG	NG	NG	NG
<i>P sp.</i>	Unknown 4	VLS	Te Puke	kiwifruit microflora	Kiwifruit	G	NG	NG	NG	NG	NG	NG	NG
<i>Ps congelans strain</i>	Unknown 5	VLS	Te Puke	kiwifruit microflora	Kiwifruit	G	NG	NG	NG	NG	NG	NG	NG
<i>Stenotrophomonas chelatiphaga</i>	Unknown 6	VLS	Te Puke	kiwifruit microflora	Kiwifruit	G	NG	NG	NG	NG	G	NG	NG
<i>P s pv. Syringae B728a</i>	Unknown 7	VLS	Te Puke	kiwifruit microflora	Kiwifruit	G	NG	NG	NG	NG	NG	NG	NG
<i>P s (van hall 1902)</i>	ICMP 11292	Landcare	New Zealand	Budrot	Kiwifruit	G	NG	NG	NG	NG	NG	NG	NG
<i>P s (van hall 1902)</i>	ICMP 11164	Landcare	Katikati	Budrot	Kiwifruit	G	NG	NG	NG	NG	NG	NG	NG
<i>P fluorescens</i>	RT198	VLS	Te Puke	kiwifruit microflora	Kiwifruit	G	G	G	NG	NG	G	NG	NG

Table 8. Copper and streptomycin resistance testing. Key: G=Growth NG= No Growth.
¹(Burkholder 1930) Dowson 1939

	Psa-V ICMP 18800 High humidity	<i>Pseudomonas sp (formerly viridiflava)</i> ICMP 13303 High Humidity
Time Interval	Psa (cfu/mL) Bottom surface of the leaf inoculated **	Psa (cfu/mL) Bottom surface of the leaf inoculated
Bacterial solution before application on leaf surface	5×10^7	2.9×10^7
Expected Total Recovery at 0 hrs	$*4 \times 10^5$	2.6×10^6
Recovery at 2hrs	1.7×10^5	1.43×10^5
Recovery at 6 hrs	2.4×10^5	N/A
Recovery at 24 hrs	1.9×10^6	2.3×10^7
Recovery at 48 hrs	2.0×10^6	1.56×10^8
Recovery at 3 days	N/A	8.1×10^7
Recovery at 5 days	4.5×10^7	N/A
Recovery at 7 days	6.2×10^6	N/A
Recovery at 8 days	N/A	3.3×10^7
Recovery at 11 days	N/A	1.2×10^6

Table 9. Bacterial survival post-inoculation onto undersides of Hayward leaves.

* Psa solution + 0.5 % Pulse® used to inoculate the bottom surface of the leaf

**Average results of 3 dilutions and plating

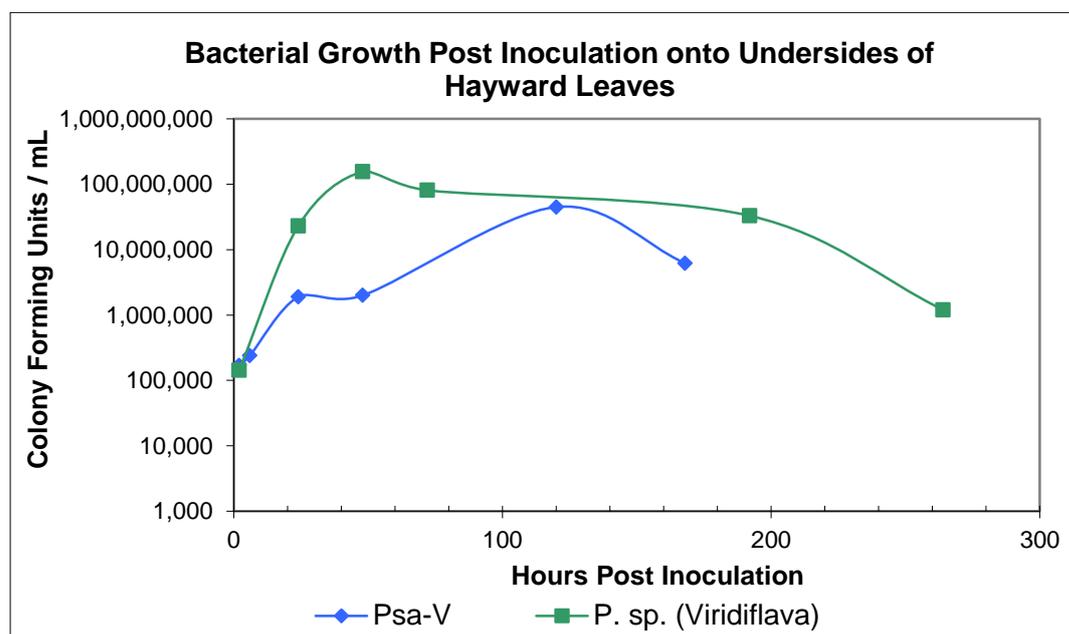


Figure 46. Survival of Psa-V and *Pseudomonas sp (formerly viridiflava)* at high humidity on the bottom surface of Hayward leaves. No plant symptoms were observed from these bacteria.

Sample collection, PCR identification and Culture Quantification

The first samples were collected on the 8th Oct from unsprayed vines. Leaves and buds with different symptoms were collected tested for Psa via qPCR and also plated on a Psa specific media, Kings B and TSA.

No	Sample type	Description	PCR Detection	PCR result Cq	Culture	Growth score	Culture quantification	P.sp Virid.	P.sp
1	Leaf	Necrotic spot	Detected	23.48	Growth	75% growth	N/A	nil	nil
2	Bud	Necrotic spot	Detected	22.54	Growth	75% growth	N/A	nil	nil
3	Leaf	Necrotic spot	Detected	22.05	Non-viable	NG	N/A	NG	NG
4	Bud	Necrotic spot	Detected	30.72	Non-Viable	NG	N/A	NG	NG
5	Leaf	Clean	ND	N/A	No Growth	NG	N/A	nil	nil
6	Bud	Clean	ND	N/A	No Growth	NG	N/A	nil	nil
7	Leaf	Clean	ND	N/A	No Growth	NG	N/A	nil	nil
8	Bud	Clean	ND	N/A	No Growth	NG	N/A	nil	nil
9	Leaf	Necrotic spot	Detected	15.63	Growth	100% growth	7.2×10^7 cfu/mL	nil	nil
10	Bud	Browned buds	Detected	15.58	Growth	100% growth	3×10^7 cfu/mL	nil	nil
11	Bud	White ooze	Detected	21.08	Growth	100% growth	3×10^6 cfu/mL	nil	nil

Table 10. Bacteria from buds pre-treatment.

These first sample results reconfirm what we have now come to expect – where we do not see some form of symptomatic necrosis we do not generally find Psa. In addition to Psa from necrotic spots on leaves and buds we did find Psa in some “white ooze”. Note that some “white ooze” may be a very highly concentrated exudate of Psa bacteria whereas in other cases it can be hard to find any Psa (some oozes are whiter than others!). In this case Psa was found at a high level (Cq = 21.08 with a quantification 3×10^6 cfu/mL). The lowest Cq value (highest level detected by qPCR) was a browned bud with Cq=15.58 (culture quantification: 3×10^7 cfu/mL). We did not find the other likely *culprits* for budrot i.e. no P. sp or Pss.

Sampling was repeated on 21st Oct after the treatments had been applied and shortly before flowering (bees in at \approx 10% flower opening on the 30th Oct).

While samples were taken from a range of treatments unfortunately this information was not transferred into the laboratory system so while the results are similar to those above we have no information as to whether the differences discussed below were associated with a particular treatment.

Results from this sampling are shown in Table 11. Again bacteria were generally only found where necrosis was obvious except in 4 cases (samples 10, 16, 17, and 21) where Psa was detected on tissues without obvious Psa symptoms. Samples 16 and 17 had quite low Psa levels but samples 10 and 21 were both very high. Overall quantifications determined by culture or from the Cq value from qPCR were lower in this sample than in the first i.e. it would appear that Psa levels were generally lower than at the first sampling. Again no Pss or P. sp were identified morphologically in cultures despite looking especially where Psa had been identified.

Samples were also sent to the University of Otago for them to assess the bacterial populations to try and clarify whether Psa or other bacteria were present. They extracted sepals from a range of buds with varying levels of browning and plated onto modified Kings B in duplicate. As with our samples those from “clear” buds had very few bacteria whereas those with any browning had *many* to *very many* and those that were withered had massive amounts of bacteria. Colonies were purified and streaked onto test media. Many of the colonies looked like Psa. Psa was extracted and 3 PCR reactions were undertaken with each DNA sample. They were all bacteria, and all pseudomonads. Those that looked like Psa proved to be so. Any of the buds with signs of browning carried a lot of Psa - certainly more than 10^8 cfu per bud.



Figure 47. Culture quantification of leaf tissue infected with Psa

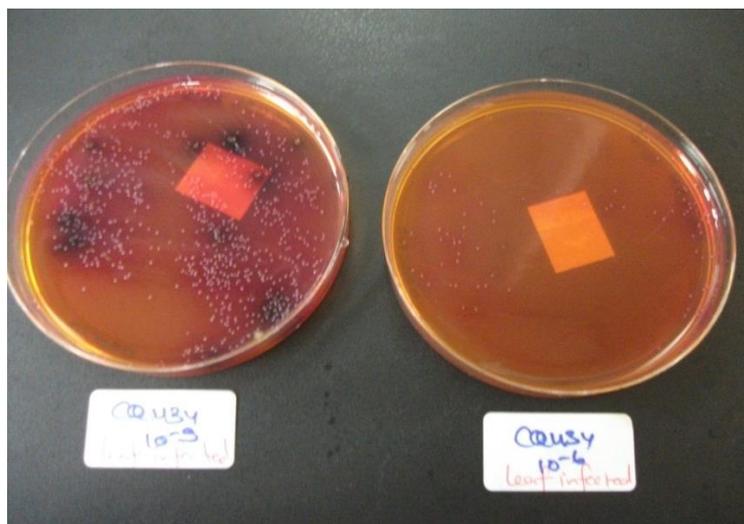


Figure 48. Culture quantification of leaf tissue infected with Psa-V

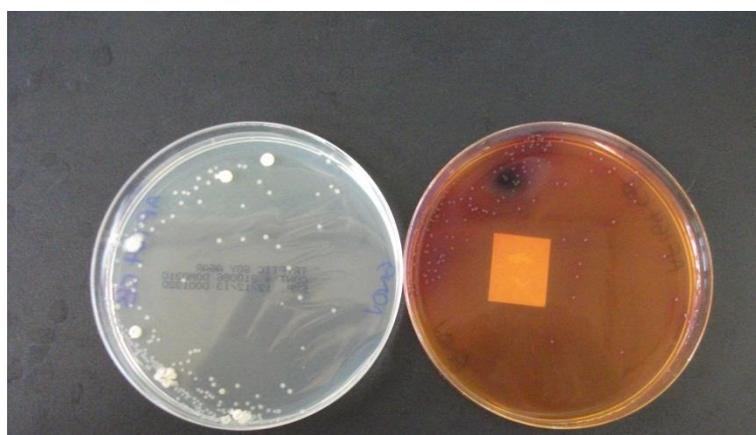


Figure 49. Culture: plates streaked from infected shoot sample on TSA plate (left) and Aitken media (right). Only Psa-V was isolated on the Aitken media (pink colonies) but the TSA agar also had other colonies.

No	Sample type	Description	PCR detection	PCR result Cq	Method	Bacterial load	Identification
1	bud	Infected	Detected	31.04	Culture	100% growth	Psa-V
2	Leaf	Infected	Detected	24.3	Culture Quantification	4 x 10 ⁵	Psa-V
3	Leaf	Non-infected	ND	N/A	non-Psa culture	Other bacteria	Bacillus cereus
4	bud	Non-infected	ND	N/A	non-Psa culture	Other bacteria	Bacillus subtilis
5	Leaf	Infected	Detected	30.18	Culture	100% growth	Psa-V
6	Bud	Infected	Detected	26.21	Culture quantification	1.6 x 10 ⁶	Psa-V
7	Cane 1	Cane 1	ND	37.13	No culture		
8	Cane 2	Cane 2	ND	36.3	No culture		
9	Cane 3	Cane 3	ND	N/A	No culture		
10	Leaf	Non-infected	Detected	22.62	Culture quantification	3 x 10 ⁶	Psa-V
11	Leaf	Non-infected	ND	N/A	non-Psa culture		
12	Bud	Non-infected	ND	N/A	non-Psa culture	Other bacteria	Bacillus cereus
13	Cane 1	Non-infected	ND	N/A	non-Psa culture		
14	Cane 2	Non-infected	ND	N/A	No culture		
15	Cane 3	Non-infected	ND	N/A	No culture		
16	Leaf	Infected	Detected	32.69	Culture quantification	4 x 10 ²	Psa-V
17	Bud	Infected	Detected	26.81	Culture quantification	2 x 10 ³	Psa-V
18	Cane 1	Cane 1	ND	32.8	No culture		
19	Cane 2	Cane 2	ND	37.52	No culture		
20	Cane 3	Cane 3	ND	N/A	No culture		
21	Bud	Bud	Detected	24.27	Culture quantification	1 x 10 ⁶	Psa-V

Table 11. Bacteria from post-treatment application.

Summary

Ambitious® effects

1. Ambitious appeared to effect the plant process which was occurring at the time of its application. Consequently different effects were observed on different orchards and on different varieties. Commonly observed effects included
 - a. Redness on developing leaves and stems
 - b. Larger diameter (but sometimes shorter) fruit stalks and larger diameter leaf petioles
 - c. Thicker leaves
 - d. Larger hanging buds
 - e. Slightly extended flowering with more later and deformed flowers
 - f. Hairier fruit which was sometimes gingery in colour
 - g. Water loss was higher from these “ginga” fruit than from visually unaffected fruit
 - h. At this stage we have not seen consistent increases in fruit size
 - i. We have not seen increased water loss in storage



Budrot

2. The observed “budrot” was not associated with wet rotting. In all cases, and through wet weather, the buds stalks and flowers were simply brown and, in severe cases, desiccated.
 - b. Browned flowers and buds remained long after flowering and when they finally dropped the stalk persist even longer as a thin brown thread.

Microbiology

3. We have characterised the plate morphology of several Psa strains, *P viridiflava*, and *P sp* on several media which provide distinguishing characteristics for positive identification.
4. Partial sequencing of the 2 strains originally associated with budrot raised more questions than answers – they did not match any sequences in Genbank
5. None of the strains of Psa showed any copper or streptomycin resistance which should mean that current Psa spray programmes should have efficacy against these bacteria
6. The *Pseudomonas sp (viridiflava)* showed resistance to copper at Cu = 0.32 mM and streptomycin at 1µg/mL.
7. Psa-V and *P sp* were grown on the underside of HW leaves and populations flourished over 7-11 days without any plant symptoms becoming evident. Population trends showed that *Pseudomonas sp (viridiflava)* grows faster than Psa-V to reach a maximum ≈48 hrs post inoculation. Psa-V took ≈5 days to reach its maximum population under the same conditions.
8. Both HW and G14 samples collected pre- and post-treatments had high Psa counts with no traces of the other 2 budrot associated bacteria.

Bacterial cause of Psa

9. In all cases from slight browning of sepals through to complete desiccation of the hanging buds we found very high numbers of Psa.
10. Conversely we rarely found high Psa numbers on buds with no browning.
 - a. Even if Psa is responsible for browning we might expect to find occasionally normal buds with Psa populations if the bacteria had only recently arrived on the surface.
11. We did not find evidence of other bacteria traditionally associated with budrot.
 - a. We acknowledge that in cases of multiple bacteria in very unequal proportions it may be very hard to find those of very low numbers
12. The observed “budrot” was not associated with wet rotting. In all cases, and through wet weather, the buds stalks and flowers simply brown and desiccate.
 - a. Browned flowers and buds remained long after flowering and when they finally dropped the stalk persist even longer as a thin brown thread.
13. The rainfall and KVH risk index showed significant risk associated with the rainfall in the period from the 19-25th Sep which was 12 days prior to the spotting we observed.
14. After periods of rainfall (Figure 20) buds could be found with white deposits remaining on the drip point (Figure 50). These may well be associated with Psa as very similar material has been collected previously and found to contain high levels of Psa.





Figure 50. White deposits on the drip point of buds after rainfall

Spray efficacy

15. The site had been severely affected by budrot in the previous season and this was reflected in variability across the block both visually and in our assessments.
16. Our experience over the first few days showed
 - a. Very rapid development from no symptoms to extreme symptoms
 - b. Areas of bud rot were seemingly scattered randomly over the block although there appeared to be more in the lower areas where damage had been worse in the previous season.
 - c. Symptoms were not assessed in relation to variety of male they were adjacent to. In the previous year males, especially M91, succumbed to Psa leaving more M33 in the block and significant numbers of missing vines in the male rows.
 - d. These symptoms stopped spreading when we appeared to be on the brink of major widespread budrot.
 - e. The spread, and cessation of spread, occurred before the treatments in this trial were applied.
 - f. We are puzzled by the rapid cessation of symptom development since weather conditions were cool and wet and it occurred even on our control plots which received no treatment.
17. This rapid spread of Psa symptoms immediately prior to the trial being laid down occurred 12 days after significant rainfall when the KVH risk index was high (Figure 6).
 - a. It remains a puzzle as to why the symptoms ceased to spread this season since at this time (7th Oct onwards) we again entered a period of high risk.
 - b. It is also a puzzle why the previous season had such a serious loss since it had significantly less rain (and consequently risk) than we experienced this season (Fig 6).
18. Vines were assessed for the extent and severity of leaf spotting as well as for bud browning
 - a. The extent of spotting was worse at the ends of canes adjacent to male rows

- b. This trend was less obvious for severity of spotting and not apparent in relation to bud browning
 - c. We did not assess symptoms in relation to local canopy density.
 - d. The extent and severity of spotting was not related to Psa treatment
 - ii. But it did vary by position (row) across the block) i.e. we hypothesise it was affected by microclimate and perhaps previous infection levels (presumably also affected by microclimate)
 - e. Bud browning was related to treatment (Table 7) and varied from 5.7 to 21.3%.
 - i. These losses may have been underestimated because of the way they were characterised although the fact that cosmetic loss was not related to treatment increases our confidence in the validity of our measurements.
19. The variability in symptoms day-to-day suggests future trials should endeavour to assess efficacy more frequently after treatment application.
- a. The relative efficacy of treatments may become more apparent if this can be undertaken

Acknowledgements

We would like to thank the Orchardist Graeme McKenzie for allowing us to undertake this trial on his property. Graeme, along with his manager Phil Young, provided us with the necessary orchard information to complete this trial. Tayah Ryan (FruitFed) kindly loaned us a mechanised sprayer for applying the treatments and assisted in the initial trial layout and treatment application.

We would also like to thank the Otago University team for assisting in the sequencing work and isolation of bacteria on leaf tissue.