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Effect of girdling on commercial cultivars in relation to *Pseudomonas syringae* pv. *actinidiae* (Psa-V) VI1389

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February 2014

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Executive summary

Effect of girdling on commercial cultivars in relation to *Pseudomonas syringae* pv. *actinidiae* (Psa-V)

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Girdling of 'Hayward' and 'Zesy002' (commonly known as Gold3) orchards was undertaken in the season of 2012-13. Girdling treatments were imposed on these vines and observations were made for leaf and secondary symptoms caused by Psa-V on the vine. At the time of girdling, cores were taken from a subset of the vines treated to establish a baseline incidence of Psa within the vines in the experimental area. At the conclusion of the trial, vines were cored for DNA analysis to determine whether Psa was present. A 'Zesy003' (Gold9) orchard was also observed; however, no baseline Psa incidence was recorded. Girdling was undertaken by the orchardist and assessments for the presence of Psa were performed post-girdling. Results for this work indicate the vines in which the girdles heal are less likely to be infected by Psa than vines where girdling has been too deep and the wounds have not healed. In non-healed girdles, Psa was generally present. Care in the course of girdling is important so the wounds heal rapidly and Psa infection via this entry point is minimised.

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1 Introduction

Girdling is a fundamental practice in many orchards. Trunk and cane girdling is one orchard operation that has been shown to improve the taste of the kiwifruit. Girdling consists of 'ring-barking' or removing a narrow strip of bark and cortical tissue from the circumference of the trunk (cordon) or the cane. The plant sugars are subsequently directed to the growing fruit rather than the roots. Girdling is used at two different times in the season, spring and autumn. Spring girdling is often used to increase the fruit size, while autumnal girdling can help to increase dry matter. Girdling results in fruit that are larger and have enhanced flavour. The orchard practice of girdling is a skill which, when applied properly, can reap rewards for growers.

There has been some criticism of this practice in recent years because of the potential of this wound to be a site of entry for the virulent strain of *Pseudomonas syringae* pv. *actinidiae* (Psa-V). When girdles are applied incorrectly, particularly if applied too deeply and cut into the xylem, there can be consequences for the vine's health. In 2011, for example, some girdles were poorly applied and did increase Psa infection in the vines, particularly in young scions that were girdled too deeply. As a result, there is now heightened awareness of the need for properly applied girdles.

A recent publication (Kiwiflier 2013) suggests that "there is little evidence to suggest that properly applied [girdles] have increased Psa infection levels in producing orchards" within the Bay of Plenty region since the Psa incursion. It is recommended that in a Psa environment girdles should be applied in periods of prolonged fine weather. Good hygiene practices are vitally important when undertaking this practice. Because of the Psa-infective environment since the outbreak of Psa, it is necessary to apply multiple protectant copper sprays whilst the girdles heal.

Because of the ongoing risk of infection by Psa and the potential for girdles to be an entry point for the bacteria, further in-field data were needed to evaluate variables associated with kiwifruit trunk-girdling.

2 Materials and methods

2.1 Orchard sites

Two sites in the Bay of Plenty region, which is heavily infected with Psa-V, were selected: 1. Katoa Orchard at Te Puke on *Actinidia deliciosa* 'Hayward' and. 2. Ngai Tukairangi Orchard at Matapihi on *A. chinensis* 'Zesy002' (commonly known as Gold3). Vines were randomly chosen on each of the orchards, with four treatments applied to 'Hayward' and three applied to Gold3. Chain girdling was undertaken on the vines at these orchards

Data were obtained from a third orchard (Taupiro Road Orchard at Katikati) during the course of a project investigating the effect of covered structures on the progression of Psa-V in that orchard (Casonato et al. 2013). Vines of *A. chinensis* 'Zesy003' (commonly known as Gold9) in that trial were chain girdled by the orchardist in February 2013. This site was formally part of the VI11451 girdling project, and thus no baseline pre-girdling information was obtained. Only post-girdling data are available for this orchard.

2.2 Girdling procedure

Girdling was undertaken on the 'Hayward' and Gold3 vines using a length of chainsaw blade with handles on each end. Two chains were used in alternation to make the girdles. After each use, the chain was washed in soapy water to remove excess material and then soaked in 80% methylated spirits for 2 minutes while the next girdle was being made. After girdling, the wound was sprayed with copper (Nordox 75 WG 1.1 g product/ L). The spring treatment girdles at the 'Hayward' orchard were sprayed both on the day of girdling and on the following day because of inclement weather.

2.2.1 'Hayward' orchard treatments

In the 'Hayward' orchard, the treatments applied were: girdling in the spring only, girdling in the autumn only, girdling at both times, and ungirdled. The girdle was made on the scion. There were 40 vines per treatment, with a total of 160 vines. The treatments and their timings were:

1. Spring girdle on 12 December 2012

2. Autumn girdle on 12 February 2013

3. Spring girdle (12 December 2012) and autumn girdle (12 February 2012)

4. No girdling – the control.

The trial layout at the 'Hayward' orchard is shown in Figure 1.

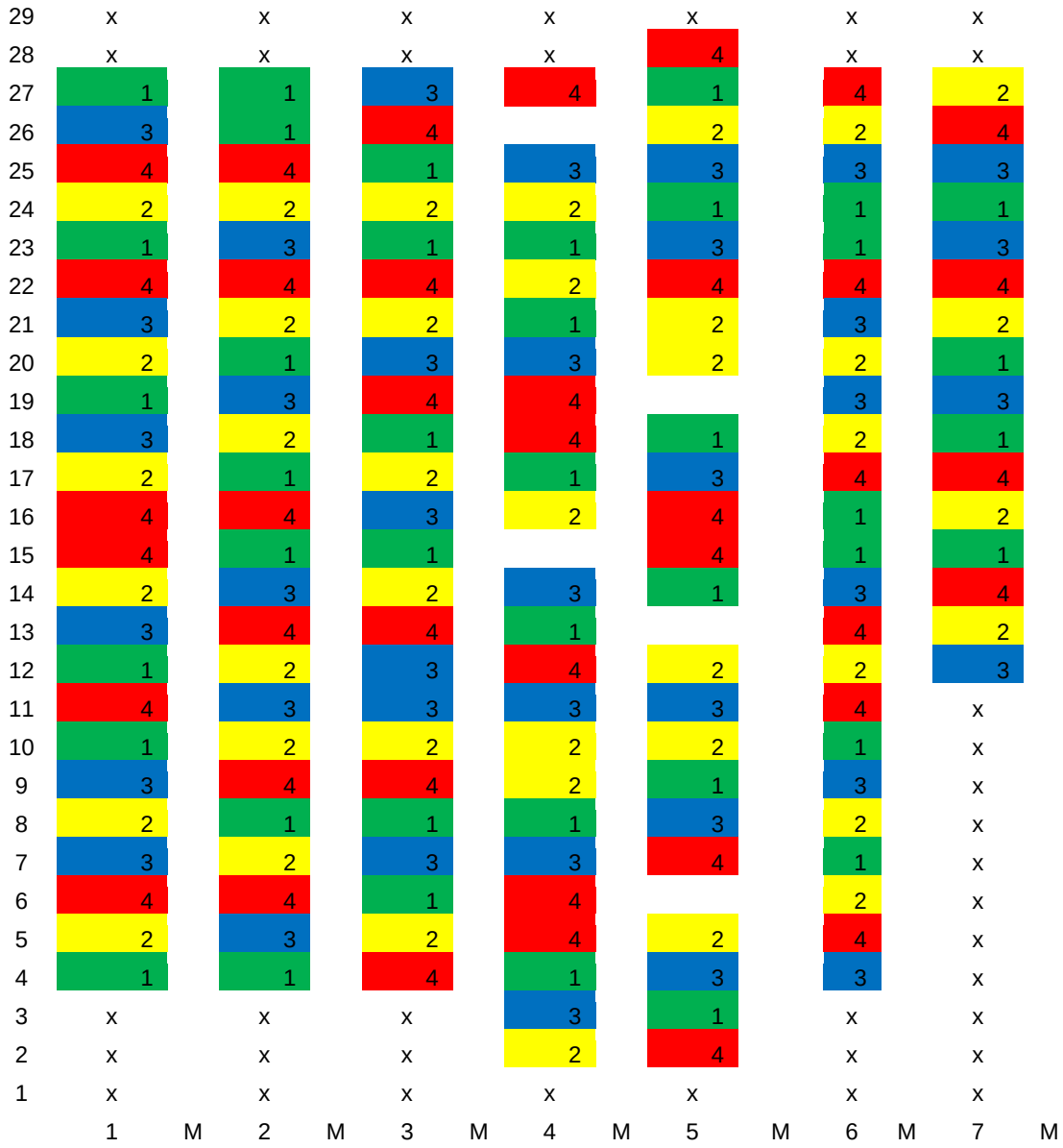

Figure 1. The layout of the *Pseudomonas syringae* pv. *actinidiae* (Psa) girdling trial on 'Hayward' kiwifruit vines. 1 (Green) = control vines with no girdling, 2 (Yellow) = spring girdle, 3 (Blue) = summer girdle, 4 (Red) = spring and summer girdle

2.2.2 Gold3 orchard

In the Gold3 orchard, the three treatments were:

1. Autumn girdling on the rootstock
2. Autumn girdling on the scion
3. No girdling – the control.

Girdles were made on either the rootstock or the scion in autumn, on 8 February 2013. There were 40 vines for each treatment, giving a total of 120 vines in the trial. The layout of the trial is shown in Figure 2. Girdling was made with a chain for rows 1, 2, 3, 4 and 13. However, as it was difficult to girdle with the chain at this site because of the difficulty of recognising when the hardwood was reached, a girdling knife was used for the rest of the vines.

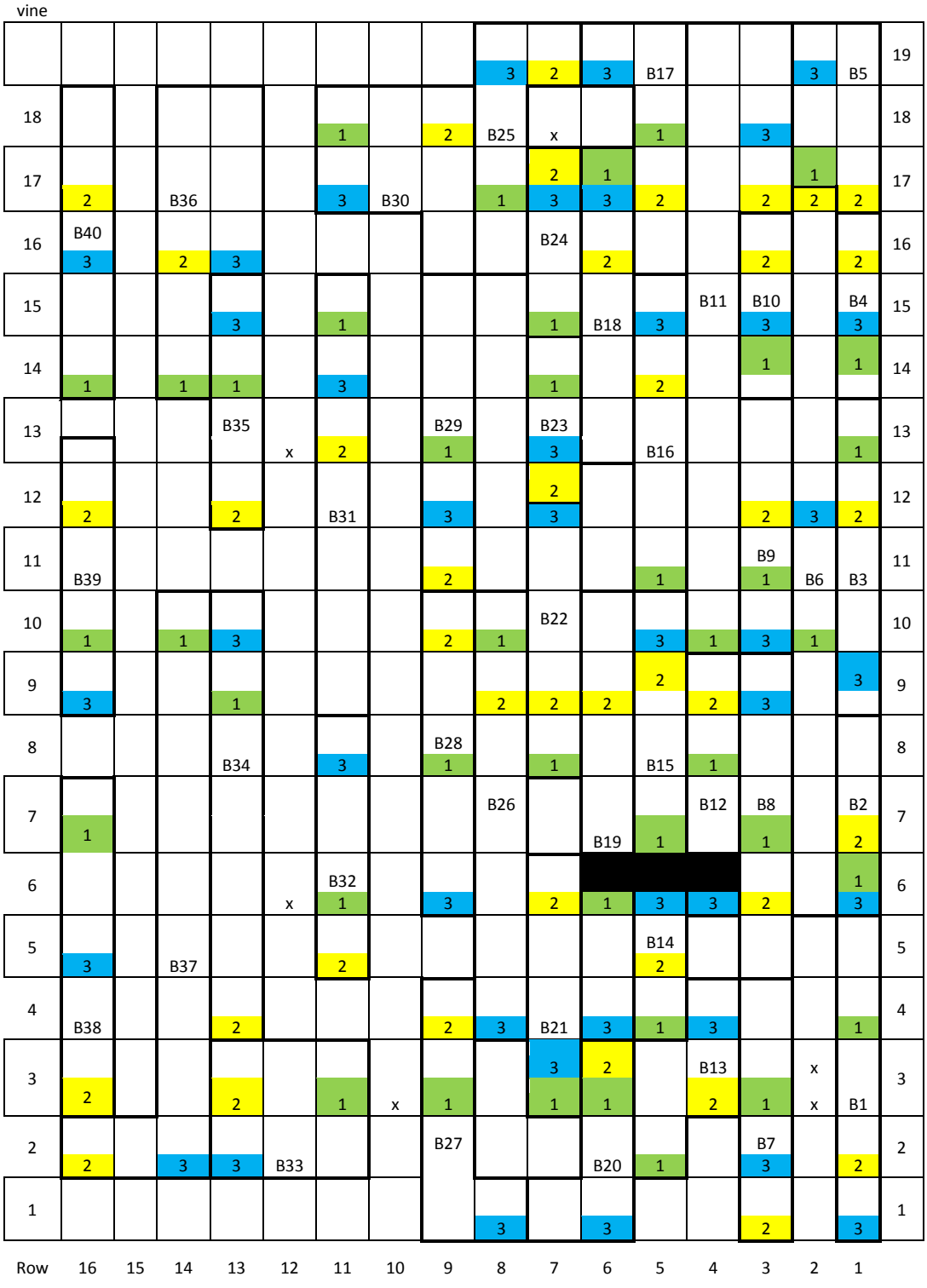

Figure 2. The block layout of the *Pseudomonas syringae* pv. *actinidiae* (Psa) girdling trial on 'Zesy002' (commonly known as Gold3) kiwifruit vines. 1 (Green) = control vines with no girdling, 2 (Yellow) = scion trunk girdle, 3 (Blue) = rootstock trunk girdle.

2.3 Plating and DNA detection of Psa

2.3.1 Sampling at the time of girdling

To ascertain baseline incidence of Psa in the tissue in close proximity to where girdles were to be made, a 0.5-mm diameter core was obtained. After coring, the hole was sprayed with copper (Nordox 75 WG 1.1 g product/ L) and back-filled with petroleum jelly. The core was split into xylem and phloem, where possible. Samples were placed directly into bacterial saline. Forty cores were taken at the 'Hayward' orchard and 30 from the Gold3 orchard. Samples from the 'Hayward' orchard were obtained on 12 December 2012 and at the Gold3 orchard on 8 February 2013.

In the laboratory, the core material was vortexed and 100 µL of solution was plated onto King's B media (King et al.1954) or King's B–C media (Mohan and Schaad 1987). Organisms that appeared to be Psa-V like were either subcultured, or a direct DNA extraction was made on the plate contents. In the methods used, the PSA was enriched by subculturing methods. DNA extraction used a boiling process and ethylenediaminetetraacetic acid (EDTA). The resultant DNA was amplified using Psa-V specific primers (Rees-George et al. 2010) in a qualitative polymerase chain reaction (PCR).

2.3.2 Sampling and post-girdling

To detect the presence of Psa after treatment, a core of 0.5-mm diameter was taken approximately 5 cm above and below the girdle. After coring, the hole was sprayed with copper (Nordox 75 WG 1.1 g product/ L) and back-filled with petroleum jelly. The whole core was placed directly into a sterile tube containing bacterial saline. Cores were taken on 21 October 2013 at the Gold3 orchard and 29 October 2013 at the 'Hayward' orchard. In total 66 Gold3 and 80 'Hayward' samples were processed as above for detecting Psa, using real-time PCR.

At the Gold9 orchard, all the vines were removed from the site during May 2013 because of the amount of Psa infection that developed in the block. Whole vine cordons, which included the girdle, were taken from the orchard to the Te Puke Research Orchard for further processing. Processing of the vines began on 24 May 2013. Samples were obtained using two processes: a core of 0.5 mm was taken approximately 5 cm above and below the girdle and a "slice" of wood approximately the same area of the coring was taken. Both samples were placed into separate tubes containing bacterial saline. In total 668 samples from Gold9 were processed as above for analysis with real-time PCR.

2.4 Observations

Assessments for secondary symptoms associated with Psa infection and leaf spots were made at the 'Hayward' and Gold3 orchards. The wood tissue symptoms were categorised on a 0–2 scale, with 0 = no dieback caused from Psa; 1 = dieback and/or ooze only in the new canes growing from the leader, and 2 = dieback and/or ooze in the leader. Where canopy was present, it was assessed for the presence of leaf symptoms (leaf spotting). A scale used by Horner & Manning (2011) was used, in which 0 = no leaf spots, 1 = <1% of leaves with spots, 2 = 2–5%, 3 = 6–15%, 4 = 16–50%, 5 = >50%.

An assessment of the girdles was also made to determine whether they were healed. A record of whether there was any visible "ooze" above or below the girdle was taken.

3 Results

The weather was a major factor in this trial, with the spring and summer period over the 2012–13 season being exceptionally dry (Figure 3). When the girdling was performed in December 2012 and February 2013, there was little rainfall. The dry weather aided in the healing of girdles and limited the dispersal of the bacteria. This weather pattern was atypical and in fact this was one of the driest years on record.

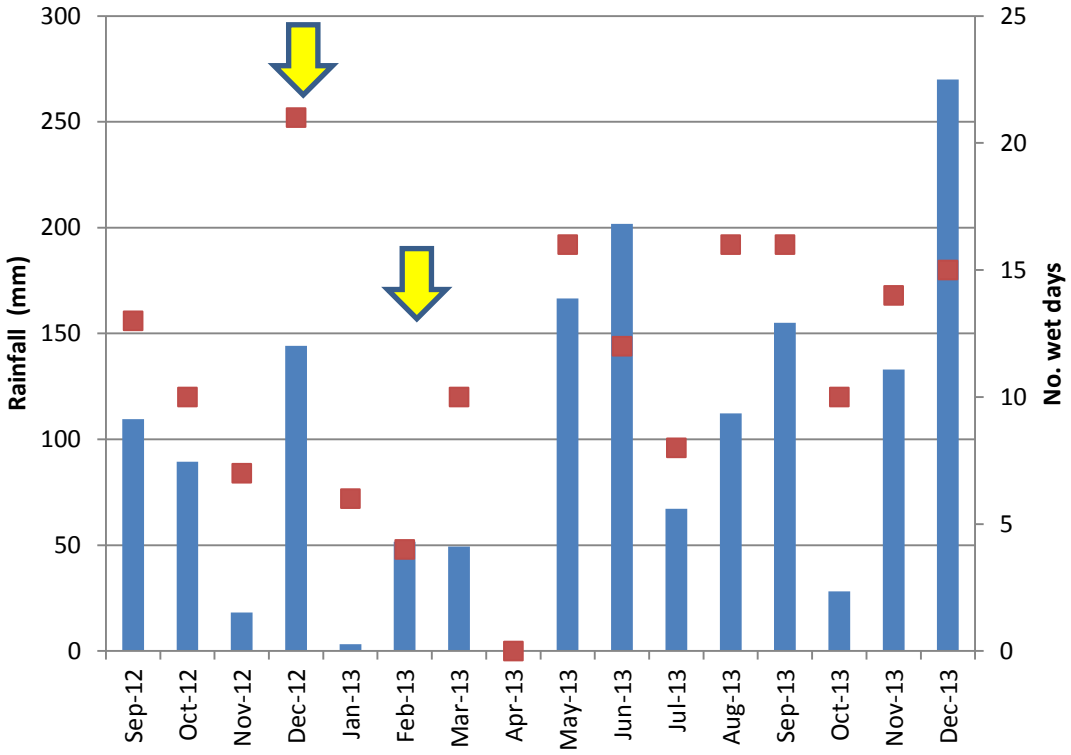

Figure 3. 2012–13 rainfall at the Te Puke Research Orchard, which is in the zone of the 'Zesy002' (Gold3) and 'Hayward' kiwifruit orchards for the *Pseudomonas syringae* pv. *actinidiae* (Psa) girdling trial. The solid blue bars indicate the amount of rainfall (mm), the red square indicates the number of rain events for the month, and the solid yellow arrow represents the time of girdling for the trial.

Results for the real time PCR are reported in this report as strong positive when the crossing threshold (Ct) was less than 30 cycles, as weak positives when the Ct was 30-35 cycles, and as negative when the Ct was greater than 35 cycles. The melting temperature was also used to confirm that the amplified product was Psa.

3.1 Gold3 orchard

3.1.1 Observations: girdles, secondary symptoms and leaves

Girdles at the Gold3 orchard healed well after girdling and subsequent coring for DNA samples (Figure 4).

No secondary symptoms, which include dieback of canes and oozing, were observed in either canes or leaders at the Gold3 orchard. There were limited leaf symptoms at this orchard, with only 11 of the 306 bays having leaf spots. Of those, 10 had less than 1% of the bay exhibiting leaf spotting symptoms and one of the 11 had between 2 and 5% of the bay exhibiting leaf spots.

Figure 4. Healed girdle at the 'Zesy002' (Gold3) kiwifruit orchard, with the coring wound above the girdle. Both the girdle and the coring wound have callused, with the coring wound still having visible petroleum jelly.

3.1.2 Plating and DNA analysis

When samples were plated onto media, no Psa-V colonies could be recognised on the plates. To confirm that no Psa was present, the samples in the bacterial saline were directly tested using molecular means. We were unable to detect the presence of any Psa from the bacterial saline containing the cored wood using the Psa-V-specific primers. All results from this orchard have a Ct value greater than 35. Thus all samples for baseline and post-girdling Psa at the Gold3 orchard were negative.

3.2 'Hayward' orchard

3.2.1 Observations: girdles, secondary symptoms and leaves

No dieback or ooze was observed in the leaders at the 'Hayward' orchard. Secondary symptoms were observed in the canes coming off the leader. Of the 390 bays observed, 28 had a cane exhibiting symptoms of dieback potentially caused by Psa-V.

Leaf spotting (Figure 5) was minimal at the 'Hayward' orchard. The majority of the bays had 5–15% of the leaves exhibiting leaf spotting during the spring of 2013. There were no differences observed in the trials after girdling. Of the 390 bays observed on 20 December 2012, only four had no leaf spotting symptoms. Of the remaining 386 bays, 290 bays had leaf symptoms expressing 1% or less of the leaves with leaf spotting, and the rest had 2-5% of the bay expressing leaf symptoms. By 20 February 2013, 387 of the total of 390 bays were expressing leaf symptoms caused by Psa-V. In one of the bays dead leaves were observed, but these were attributed to natural death of the vine and not to Psa-V. Table 1 shows the incidence of leaf spot caused by Psa-V on 20 February 2013.

Figure 5. Leaf spotting on a 'Hayward' leaf at an orchard in the Bay of Plenty.

Table 1. The incidence of leaf spotting in the *Pseudomonas syringae* pv. *actinidiae* (Psa) girdling trial bays in the 'Hayward' orchard on 20 February 2013

Leaf spot symptoms in a bay	Total % trial bays affected
Less than 1%	12
2-5%	45
6-15%	36
16-50%	6
Greater than 50%	1

All girdles, except one, healed within 2 months (Figure 6). The one girdle that did not initially heal did so later. A sample from the girdle was taken to check for the presence of Psa using molecular techniques and was found to be negative for Psa. It is possible this girdle was slightly deeper than the others, hence the greater time required for healing. Samples where the core was taken for DNA analysis healed well.

Figure 6. Healed girdle and core on 'Hayward' vine.

3.2.2 Plating and DNA analysis

None of the samples taken at the time of girdling indicated the presence of Psa in the 'Hayward' orchard with all Ct values being greater than 35 cycles. After girdling there was, at the time of coring, weak positive results in seven of the 40 samples (Table 2). The 7 samples all have a Ct value between 30–35 cycles. Weak positives were found above and below the girdle and in all treatments, including the control.
One weak positive result was found in a sample taken at the same site as the baseline data (Table 2). There were no positive results that would suggest high concentration of Psa in the sample which had been extracted from an agar plate after enrichment of the sample. The remaining 33 samples tested were negative for the presence of Psa with Ct values greater than 35 cycles (Table 2).

Table 2. Core samples taken below and above the girdle on 'Hayward' kiwifruit orchards at Katoa after girdling in spring, summer, spring + summer, or no girdling, the control during the 2012–2013 season. A negative result was obtained with a crossing threshold of less than 30 cycles, a weak positive had a crossing threshold of between 30–35 cycles and a negative results indicated a crossing threshold of greater than 35 cycles. The grey pixilation indicates the samples where baseline and post-girdling cores were taken.

Treatment	Above the girdle	Below the girdle
Control	Weak positive	Negative
Control	Negative	Negative
Control	Weak positive	Negative
Control	Negative	Negative
Control	Negative	Weak positive
Spring	Negative	Weak positive
Spring	Negative	Negative
Spring	Negative	Negative
Spring	Negative	Negative
Spring	Negative	Negative
Summer	Negative	Weak positive
Summer	Negative	Negative
Summer	Negative	Negative
Summer	Negative	Negative
Summer	Negative	Weak positive
Spring and Summer	Weak positive	Negative
Spring and Summer	Negative	Negative
Spring and Summer	Negative	Negative
Spring and Summer	Negative	Negative
Spring and Summer	Negative	Negative

3.3 Gold9 orchard

3.3.1 Observations: girdles, secondary symptoms and leaves

This orchard had considerable infection caused by Psa. There were extensive woody tissue symptoms, which included dieback of canes and oozing from the wood (Figure 7).

Figure 7. *Pseudomonas syringae* pv. *actinidiae* (Psa-V) secondary symptoms with visible ooze in *Actinidia chinensis* 'Zesy003' (commonly known as Gold9).

Numerous girdles at the Gold9 orchard did not heal. This appeared to be related to the depth of the girdle (Figure 8). Where the girdle healed, there was often no ooze and there was the formation of abundant, healthy callus (Figure 9).

Figure 8. Unhealed *Actinidia chinensis* 'Zesy003' (commonly known as Gold9) after girdling and prior to being processed for core sampling. *Pseudomonas syringae* pv. *actinidiae* (Psa-V) ooze can be seen above the girdle on the left, with staining of the bark.

Figure 9. A healed *Actinidia chinensis* 'Zesy003' (commonly known as Gold9) girdle with no ooze expressed.

3.3.2 Plating and DNA analysis

DNA analysis indicated that many of the vines were infected with Psa, indicated with a Ct value that was less than 30 cycles (Table 3). Many of the vines had visible ooze above the girdle (Figure 10) with no visible symptoms below. In some vines, despite symptoms being visible below the girdle, Psa was detected by molecular means as there was a Ct value of greater than 35 cycles obtained (Table 3).
The presence of Psa appeared to be correlated with the healing of the girdle, whether it healed fully, partially or not at all. Healed girdles frequently had no Psa detected in the region above the girdle (Table 3) whereas partially healed girdles would often have Psa infected cores above the girdle but not below (Table 3). There were exceptions to this observation, with some healed girdles having positive detections both above and below the girdle (Table 3). Girdles that did not heal in most cases had Psa present both above and below the girdle (Table 3).

Table 3. Results of *Pseudomonas syringae* pv. *actinidiae* (Psa-V) DNA analysis post-girdling on an *Actinidia chinensis* 'Zesy003' (commonly known as Gold9) orchard in the Bay of Plenty. Cores were taken above and below the girdle. An indication to whether the vines sampled from were under a breathable cover or had a healed girdle is noted. A positive result for the presence of Psa-V DNA is indicated by a crossing threshold of less than 30 cycles, a weakly positive results when a crossing threshold of 30–35 cycles and a negative value when the crossing threshold was greater than 35 cycles.

Above girdle on left and below girdle on the right	Sample	Cover	Healed girdle	Above girdle	Below girdle
	Bay 25 Row 2/3	Yes	No	Positive	Positive
	Bay 13 Row 6	Yes	No	Positive	Positive
	Bay 23 Row 4/5 Vine 1		No	Positive	Positive
	Bay 5 Row 4/5	No	No	Positive	Positive
	Bay 22 Row 2/3 Vine 2	Yes	Yes	Negative	Negative
	Bay 6 Row 2/3	Yes	No	Positive	Positive
	Bay 20 Row 4/5 Vine 1	No	No	Positive	Negative

Above girdle on left and below girdle on the right	Sample	Cover	Healed girdle	Above girdle	Below girdle
	Bay 4 Row 4/5 Vine 2	No	Partial	Positive	Negative
	Bay 14 Row 4/5 Vine 1	Yes	No	Positive	Positive
	Bay 25 Row 2/3 Vine 2	Yes	No	Positive	Positive
	Bay 20 Row 6	No	Yes	Negative	Negative
	Bay 7 Row 6 Vine 2	No	No	Positive	Positive
	Bay 25 Row2/3 Vine 2	Yes	No	Positive	Positive

Above girdle on left and below girdle on the right	Sample	Cover	Healed girdle	Above girdle	Below girdle
	Bay 19 Row 2/3 Vine 2	No	Yes	Negative	Negative
	Bay 5 Row 1 Vine 2	Yes	No	Positive	Negative
	Bay 19 Row 4/5 Vine1	No	No	Positive	Positive
	Bay 16 R2/3 Vine 2	Yes	Yes	Negative	Negative
	Bay 3 Row 4.5	No	Yes	Negative	Negative
	Bay 7 Row 4/5	Yes	Partial	Positive	Positive
	Bay 6 R2/3	Yes	No	Positive	Positive

Above girdle on left and below girdle on the right	Sample	Cover	Healed girdle	Above girdle	Below girdle
	Bay 15 R2/3	Yes	Yes	Negative	Negative
	Bay 22 R4/5 Vine 1	Yes	Partial	Positive	Negative
	Bay 20 R4/5 Vine 2	No	No	Positive	Positive
	Bay 2 Row 6	No	No	Positive	Negative
	Bay 21 Row 2/3 Vine 1	Yes	Yes	Negative	Negative
	Bay 13 Row 4/5	Yes	Partial	Positive	Negative

Above girdle on left and below girdle on the right	Sample	Cover	Healed girdle	Above girdle	Below girdle
	Bay 6 Row 1 Girdle 1 Girdle 2 Vine 1	Yes Yes	No No	Positive Positive	Positive Positive
	Bay 2 Row 1 Girdle 1 Girdle 2 Vine 1	No No	Yes Partial	Negative Positive	Negative Positive
	Bay 5 Row 1 Girdle 1 Girdle 2 Vine 1	Yes Yes	No No	Positive Positive	Negative Weakly positive
	Bay 25 R4/5 Girdle 1 Girdle 2 Vine 1	Yes	No No	Positive Positive	Positive Positive
	Bay 13 Row 1 Girdle 1 Girdle 2	No	No Partial	Positive Positive	Negative Weakly positive
	Bay 7 Row 1 Girdle 1 Girdle 2	No	No No	Positive Positive	Positive Positive

Above girdle on left and below girdle on the right	Sample	Cover	Healed girdle	Above girdle	Below girdle
	Bay 18 Row 2/3 Girdle 1 Girdle 2 Vine 1	No	No No	Positive Positive	Positive Positive
	Bay 18 Row 2/3 Vine 2	No	No	Positive	Positive

Figure 10. An *Actinidia chinensis* 'Zesy003' (commonly known as Gold9) vine from a Bay of Plenty orchard showing a partially healed girdle. The cores were taken for DNA analysis and indicated *Pseudomonas syringae* pv. *actinidiae* (Psa-V) was present above the girdle (on the left), while no Psa was detected below the girdle (right). The orange/red staining indicates the presence of Psa-V within the vine.

4 Discussion

The work undertaken during the growing season of 2012–2013 indicated that girdling did not increase the presence of Psa in the 'Hayward' and Gold3 vines within those orchards. In the 'Hayward' and Gold3 orchards, there was no highly positive molecular detection of the presence of Psa in the samples taken. In the 'Hayward' and Gold3 orchards, the girdles were well executed and subsequently healed extremely well, developing healthy callus. Conversely, girdling did result in the expression of ooze in heavily Psa-infected vines in Gold9. In that orchard, the girdling was poorly executed, with girdles being deep, potentially cutting into the xylem in some of the vines. Psa was readily detected by molecular means in the Gold9 orchard, but there appeared to be a correlation between the presence on Psa and the extent of healing of the girdle. This project has clearly shown that when a girdle heals in dry conditions that will minimise the spread of Psa by rain splash, there is little effect on the incidence of Psa within the vine. In dry environments, the girdling wounds do not appear to facilitate the entry of Psa.

No Psa was detected in the samples taken from the Gold3 vines either before or after girdling. All crossing threshold values were over 35 cycles. At the Gold3 orchard there was an extremely low incidence of leaf spotting and no secondary symptoms in the trial vines. Thus the amount of readily available Psa inoculum in close proximity to the open wounds after girdling was negligible. There was, however, an inoculum source relatively close to the trial vines at the Gold3 orchard. In other blocks on the orchard, Psa-V infected vines expressed secondary symptoms. Even though the inoculum was present at the orchard and the trials were conducted in a Psa-V infected zone, because of the dry environmental conditions during 2012–2013, the spread of the bacteria was negligible. This project has indicated that in dry conditions when there are relatively few rain events, there is a low chance of infection via open girdling wounds.

At the 'Hayward' orchard, despite the presence of Psa in the form of leaf spotting and secondary dieback, only weakly positive results were obtained using Psa-specific primers. Weakly positive results were obtained from 7 of the 40 samples and the crossing threshold values of 30–35 cycles was obtained. These weakly positive results could indicate there was a presence of Psa in the samples although the numbers of bacteria were low. There were no strongly positive results (Ct values less than 30 cycles) obtained from the DNA analysis. This suggests that the presence of the bacteria in the wood was minimal and thus the girdling did not aid in the penetration of the bacteria. It would be useful to undertake DNA analysis of the cores from the same position again in 12 months' time, to determine whether the Psa result was then strongly positive, rather than weakly positive. A positive result may reflect a proliferation of the bacteria in that area.

The Gold9 orchard had a substantial amount of leaf spotting and secondary symptoms. Many vines had ooze prior to girdling; therefore, there was a high inoculum load in close proximity at the time of girdling the trial vines. There is no way to determine whether Psa was present in the girdling region of the sampled cores as no baseline data were obtained pre-girdling. At this orchard it is apparent that when girdles were made too deeply, they did not heal. In many of the vines when this occurred, there was a positive DNA result for the presence of Psa above and below the girdle. In girdles where there was partial healing, there was often Psa above the girdle but not below. When the girdle had fully healed there was often no Psa above and below girdle in the core taken. This orchard, in particular, highlights the need for girdling to be executed accurately and not to leave an open wound that can be readily infected from Psa that is available on the vine itself and the environment.

It is vitally important to note that the 2012–2013 growing season was very dry and few rain events occurred. Moist conditions facilitate the dispersal of Psa bacterium. Because of this, there was decreased inoculum pressure at the 'Hayward' and Gold3 orchards and thus these results may not be a true reflection of what occurs when girdling in a 'normal' Psa environment. Girdling may present an entry point for Psa inoculum; however, there are numerous other potential entry points on the vine.

Open wounds will act as an entry point for Psa but this project has shown that if the wound, in this case a girdle, is able to heal rapidly under dry conditions, then the opportunity for bacterial entry via this means is significantly reduced. Thus good hygiene of the girdle until it has healed is paramount. As this project has demonstrated, an application of copper after the girdle, and cleaning girdling implements between vines will facilitate the vine's healing and reduce the risk of infection.

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