PFR SPTS No. 12997

Monitoring effectiveness of wound protectants against Psa

Everett KR, Pushparajah IPS, Vergara M, Shahjahan K, Parry B, Casonato S

March 2016





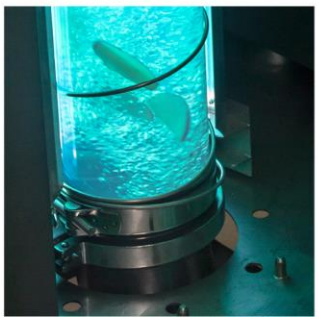













Confidential report for:
Zespri

PUBLICATION DATA

Everett KR, Pushparajah IPS, Vergara M, Shahjahan K, Parry B, Casonato S. March 2016. Monitoring effectiveness of wound protectants against Psa. A Plant & Food Research report prepared for: Zespri. Milestone No. 66873. Contract No. 31956. Job code: P/345515/01. SPTS No. 12997.

Report approved by:

Kerry Everett
Scientist, Plant Pathology
March 2016

Suvi Viljanen-Rollinson
Science Group Leader, Plant Pathology
March 2016

CONTENTS

EXECUTIVE SUMMARY

Monitoring effectiveness of wound protectants against Psa

Everett KR[1], Pushparajah IPS[1], Vergara M[1], Shahjahan K[1], Parry B[2], Casonato S
Plant & Food Research: [1]Auckland, [2]Te Puke

March 2016

Introduction

There is currently no research evidence that wound protectants applied to kiwifruit vines in the field are effective. This set of experiments was designed to test wound protectants on potted plants and kiwifruit vines in the field, to provide effective protection against infection by Psa after pruning.

Methods

Potted plants

Two-year-old Actinidia chinensis var. deliciosa 'Zes007' (also known as G11), *A. chinensis* var. *chinensis* x *A. chinensis* var. *deliciosa* 'Zesh004' (also known as G14) and *A. chinensis* var. *chinensis* 'Hort16A' kiwifruit vines in pots were placed in a greenhouse with overhead watering in a completely random block design (G11) or a Latin square (G14, 'Hort16A'). Two vines from each variety were tested for presence of Psa using qPCR and two sets of primers (F3/R4 and HopZ2b) before treatments were applied. Vines were wounded by cutting through the stem 1 m above soil level on 10 February 2015. Wound protectants were applied, then after drying overnight, vines were spray inoculated at the wound with 10^4 and 10^6 cfu/mL Psa-V. There was an uninoculated control, and an inoculated untreated control. There were 10 replicate vines per treatment.

After 11 weeks, lesions were measured and 5 mm long segments of cane taken at the wound and 5 and 10 cm below the wound for DNA extraction and qPCR analysis.

'Chieftain' vines in the orchard

Winter pruning

Eleven to sixteen-year-old *A. deliciosa* 'Chieftain' male kiwifruit vines at Te Puke Research Centre were tested for baseline levels of Psa by taking a 5 mm segment from pruned canes on 21–24 July 2015 for DNA extraction and qPCR analysis. At that time wound protectants were applied to five canes on each of 12 vines. There was an unwounded untreated control and a wounded untreated control. Two weeks after application of treatments, a 5 mm segment was taken from the cut end for DNA extraction and qPCR analysis.

Spring pruning

Several 'Chieftain' vines had been removed prior to the spring pruning trial. Five canes on six 'Chieftain' vines were treated with the same methods as for the winter pruning trial on November 19 2015. Two weeks after application of treatments, a 5 mm segment was taken from the cut end for DNA extraction and qPCR analysis.

Results

- InocBloc paste and copper paste consistently protected pruning wounds on kiwifruit vines from infection by Psa-V, in both potted plant trials and in the field.
- No other wound protectants provided statistically significant protection against Psa-V, in either potted plant trials or in the field.
- For potted plants, copper paste provided statistically significant wound protection compared with inoculated controls for G14 and 'Hort16A' when mean Ct values were analysed. It is apparent from the count data (% canes infected) that copper paste was the best wound protectant although this was not statistically significant.
- On G11 potted plants, InocBloc paste provided statistically significant wound protection compared with untreated controls for both incidence data and mean Ct values.
- On 'Chieftain' male kiwifruit vines in the field, copper paste and InocBloc paste provided statistically significant wound protection compared with controls when applied both in winter and spring, both from incidence data and from Ct values. InocBloc paste appeared to provide better protection than copper paste, although this was not statistically significant.
- Without application of wound protectants, the incidence of 'Chieftain' kiwifruit canes infected by Psa in winter increased from 3.3% to 50% in the 2 weeks after wounding, and in spring from 30% to 72%.

Conclusions and Recommendations

- It would be advisable to apply an effective wound protectant such as copper paste or InocBloc paste immediately after pruning to reduce infection of vines in the field.

For further information please contact:

Kerry Everett
Plant & Food Research Auckland
Private Bag 92169
Auckland Mail Centre
Auckland 1142
NEW ZEALAND

Tel: +64 9 925 7000
DDI: +64 9 925 7133
Fax: +64 9 925 7001
Email: kerry.everett@plantandfood.co.nz

1 INTRODUCTION

Since November 2010 (Everett et al. 2011) New Zealand kiwifruit (*Actinidia* spp.) has been subjected to a strain of *Pseudomonas syringae* pv. *actinidiae* (Psa) causing a serious disease. This strain is also known as Psa-V (McCann et al. 2013) or biovar 3 (Chapman et al. 2012; Vanneste et al. 2013). It is commonly referred to as Psa-V.

This bacterium is known to enter kiwifruit plants through wounds (Serizawa & Ichikawa 1993), as well as through natural openings such as lenticels (Everett et al. 2013b), hydathodes and stomata (Serizawa & Ichikawa 1993). Kiwifruit vines are routinely pruned in winter and in spring, providing wounds for Psa-V to infect. Although several wound protectants are being used, it is not known how effective these are.

Previously, experiments to compare the efficacy of wound protectants were conducted in the field (Miller et al. 2012) and on 'Bruno' seedlings grown in a shade house (Everett et al. 2014). In neither experiment were there significant differences between treatments. However it is possible that the concentration of Psa (10^8 cfu/ml) used in the shade house trials was simply too high for the treatments to be effective (Everett et al. 2014).

Assessments were made by measuring the brown staining that resulted from inoculations of cut stems in the latter study (Everett et al. 2014), and of naturally occurring infections in the former (Miller et al. 2012). It is possible that measuring the brown staining may not be a good indicator of the efficacy of the wound protectants as this symptom is a response by the plant to prevent infection of wounds by Psa-V. Instead, assessing the presence of Psa-V by using specific primers in the polymerase chain reaction (PCR) (Rees-George et al. 2010; Rikkerink et al. 2011) may be a better test of the success of wound protectants.

The primers of Rees-George et al. (2010) are designed to detect the ribosomal RNA inter-transcribed spacer (ITS) region of Psa, and will detect both strains present in New Zealand (Psa-V and *Pseudomonas syringae* pv. *actinidifoliorum* , commonly known as Psa-LV (Cunty et al. 2015)). Because there are five copies of the ITS region in the Psa genome (Templeton et al. 2015) these primers are very sensitive, because they amplify five times more DNA than primers designed to a single copy gene region. The primers of Rikkerink et al. (2011) are designed to a single copy gene associated with pathogenicity, and are specific to Psa-V.

This study aims to compare the efficacy of wound protectants on inoculated potted plants in late summer, then on naturally infected kiwifruit vines in the orchard in spring and winter by using qPCR (quantitative PCR) to detect Psa-V.

2 METHODS

This series of trials was conducted at The New Zealand Institute for Plant & Food Research Limited facilities in Te Puke.

2.1 Potted plant trials

Treatments were applied on 10 February 2015 to 2-year-old *Actinidia chinensis* var. *deliciosa* 'Zes007' (also known as G11) G11, *A. chinensis* var. *chinensis x A. chinensis* var. *deliciosa* 'Zesh004' (also known as G14) and Hort16A *A. chinensis var. chinensis 'Hort16A'* purchased from Waimea Nursery, Nelson. There were 10 replicate plants per each of 10 treatments for G14 and 'Hort16A', a total of 100 plants each. For G11, there were 10 replicate plants per each of eight treatments, a total of 80 plants. G11 plants were laid out in a completely randomised block design, and G14 and 'Hort16A' plants were laid out in a Latin square design in a greenhouse (Figure 1). Plants were irrigated by overhead watering.

Figure 1. Treated kiwifruit plants (*Actinidia chinensis* var. *deliciosa* 'Zes007' (also known as G11) G11, *A. chinensis* var. *chinensis* x *A. chinensis* var. *deliciosa* 'Zesh004' (also known as G14) and Hort16A *A. chinensis* var. *chinensis* 'Hort16A') in a Latin square design and a completely random block design in a greenhouse.

Plants were cut through the main stem at 1 m height with sterilised secateurs. A 5 mm long cane segment from two plants of each variety was excised from the cut edge and placed in a small plastic ziplock bag for DNA extraction and PCR tests. Secateurs were sprayed with 70% ethanol and scrubbed clean with handitowels between each cut. Immediately after cutting, pruning products were applied to the wound using the rates and application methods described in Table 1. Treatments applied to G14 and 'Hort16A' were:

1. copper paste
2. Product B
3. Product A
4. Vinevax™
5. Actigard™
6. Nanospada™ 500
7. Prunesafe™
8. Kasumin® plus penetrant (Kasumin 5ml/L plus Engulf™ 2ml/L)
9. inoculated control
10. uninoculated control

Treatments applied to G11 were:

1. InocBloc™ spray
2. InocBloc ™paste
3. InocBloc™ mastic
4. Kasumin
5. penetrant (Engulf) only
6. inoculated control
7. uninoculated control
8. Kasumin plus penetrant.

Immediately after application of the pruning products, five plants for each treatment were spray inoculated with a 10^4 cfu/ml suspension of Psa, and the remaining five plants were spray inoculated with a 10^6 cfu/mL suspension of Psa.

Table 1. Chemicals used to treat wounds.

Trade name	Active ingredient	% a.i.	Application rate	Application method
Copper sulphate pentahydrate	Copper sulphate pentahydrate	250 g/L (25%)	9.4 kg/L	Paint
Product B	Tebuconazole	10 g/L	undiluted	Paint
Product A	Tebuconazole + ochthilinone	10 g/L + 17.5 g/L	undiluted	Paint
Vinevax™	*Trichoderma atroviride*	5 x 10^8 colony forming units/g	10 g / L	Spray
Actigard™	Acibenzolar-s-methyl	500 g/kg	2 g/ L	Spray
Nanospada™ 500	Quaternary ammonium chloride	1.25%	1 L/3 L	Spray
Prunesafe	Not supplied	Not supplied	Not supplied	Paint
Product C	Tebuconazole	10 g/L	undiluted	Paint
Kasumin	Kasugamycin	20 g/L	5 ml/ L	Spray
InocBloc™ spray	Pine tar	c. 45% pine tar/c. 45% ethanol	undiluted	Spray
InocBloc™ paste	Pine tar	>90%	undiluted	Paint
InocBlock™ mastic	Pine tar	Not supplied	Not supplied	Paint
Emix	elicitors	Not supplied	Not supplied	Spray
Engulf®	Polyether-modified trisiloxante	Not supplied	2 ml/ L	Spray

Nanospada is a trademark of Katan Technologies IP Pte. Ltd. Vinevax is a trademark of Agrimm Technologies Ltd., InocBloc is a trademark of Safesan Co. Ltd, Engulf is a trademark of Etec Crop Solutions Ltd. Kasumin is a registered trademark of Hokko chemical Industry Co., Ltd. Actigard is registered to Syngenta Crop Protection Limited, Prunesafe is a trademark for Ecotek Horticulture

External lesion length was measured for every plant on 19/3/2015, and on 28/4/2015. On the 28/4/2015 a 5 mm long cane sample was taken at the wound site, then at 5 cm and 10 cm below the wound and placed in a small plastic ziplock bag for DNA extraction and PCR testing.

2.2 Mature vine trials

2.2.1 Winter pruning

Eleven to sixteen-year-old 'Chieftain' male *Actinidia deliciosa* kiwifruit vines on Block 50, Te Puke Research Centre, 412 No. 1 Road, Te Puke, were pruned with sterilised secateurs. Secateurs were sprayed with 70% ethanol and scrubbed clean with handitowels between each cut. A 5 mm long cane segment from each wound was excised from the cut edge and placed in a small plastic ziplock bag for DNA extraction and PCR tests. Immediately after cutting, pruning products were applied to the wound using the rates and application methods described in Table 1. 'Chieftain' male vines were treated on 21–24 July 2015. There were 12 vines, and 5 canes were treated on each vine with nine wound protectants (Figure 2). There was a wounded and an unwounded control. Treatments were:

1. Copper paste
2. Vinevax
3. Product B
4. Actigard
5. Emix

6. Kasumin + Engulf
7. Kasumin
8. InocBloc paste
9. Untreated wounded
10. Untreated unwounded

On 4 and 5 August a 5 mm long cane sample was taken at the wound site, then at 5 cm above the wound and placed in a small plastic ziplock bag for DNA extraction and PCR testing.

Position	Row 1	Row 2	Row 3	Row 4	Row 5	Row 6	Row 7	Row 8	Row 9	Row 10
1a	X	X			8	X	9	X	X	X
1b										
2a	X	X	X	1.1	X	X	X	X	X	X
2b		X	X		X	X		X	X	
3a	X	X	X	X	X	X	X	X	X	X
3b		X	X		X	X		X	X	
4a	X	X	X	2	X	X	10.6	X	X	X
4b		X	X		X	X		X	X	
5a	X	X	X	X	X	X	X	X	X	X
5b		X	X		X	X		X	X	
6a	X	X	X	3.2	X	X	X	X	X	X
6b		X	X		X	X		X	X	
7a	X	X	X	X	X	X	11	X	X	X
7b		X	X		X	X		X	X	
8a	X	X	X	4	X	X	X	X	X	X
8b		X	X		X	X		X	X	
9a	X	X	X	X	X	X	X	X	X	X
9b		X	X		X	X		X	X	
10a	X	X	X	5.3	X	X	12.5	X	X	X
10b		X	X		X	X		X	X	
11a	X	X	X	X	X	X	X	X	X	X
11b		X	X		X	X		X	X	
12a	X	X	X	6	X	X	X	X	X	X
12b		X	X		X	X		X	X	
13a	X	X	X	X	X	X	X.4	X	X	X
13b		X	X		X	X		X	X	
14a	X	X	X	7	X	X	X	X	X	X
14b		X	X	X		X		X	X	
15a	X				X	X	X	X	X	X
15b		X								

N

4.2m

3.5m

Figure 2. Position of *Actinidia deliciosa* 'Chieftain' male kiwifruit vines treated with wound protectants. Treated canes are denoted by green shading. The first number is for winter pruning, the second number for spring pruning. The bays are 4.2m long and there is 3.5m between the vines. North is indicated. Treated 'Chieftain' vines in row 7 were planted in 1998/99, and in the other rows in 2005. Plant X.4 was not treated in winter.

2.2.2 Spring pruning

Five canes on six 'Chieftain' vines (Figure 2) were treated with the same products and methods as for the winter pruning trial, with the addition of one more treatment (Product C). Samples for DNA extraction were removed before application of pruning products on November 19, and then after 2 weeks on 3 December, as described above.

2.3 DNA extractions

A 1000 µL aliquot of bacterial saline (BS) was added to the small ziplock bags containing cane segments. The plant material was macerated inside the bag using a pestle, then 800µl of the solution was removed and placed in a 1.5mL Eppendorf tube for DNA extraction.

The solution was centrifuged for 5 min at 8500 rpm and the resultant pellet was re-suspended in 1 mL BS, centrifuged again then re-suspended in 200 µL 0.1 mM EDTA. The Eppendorf tubes were secured with microtube cap locks, then immersed in water at 100°C for 5 min., then placed immediately on ice. After 10-15 min. on ice, the tubes were again centrifuged for 5 min. at 14,000rpm. A 1 µL aliquot of this suspension was used as a template in qPCR reactions.

2.4 Quantitative Polymerase Chain Reaction (qPCR)

2.4.1 Quantification

The 10 µL/well reaction consisted of 1 µL of DNA, 5 µL SYBR Green I Master, 4 µL GIBCO™ water and 5 µM of each forward and reverse primers (0.5 µL each), and was conducted in the LightCycler® 480 Real-Time PCR System under the following conditions: 95°C for 10 min, 45 cycles of 95°C for 5 s, 60°C for 7 s, 72°C for 7 s, followed by melting-curve analysis with a temperature profile slope from 65°C to 97°C with continuous fluorescence measurement. Psa-V was quantified using the HopZ2b primers of Rikkerink et al. (2011) and the PsaF3/R4 primers of Rees-George et al. (2010) according to the methodology developed in Everett et al. (2013a).

2.5 Dilution series with InocBloc samples

Samples from five vines treated with InocBloc in winter that were negative were tested in qPCR with both primers (F3/R4 and HopZ2b) undiluted, and diluted with GIBCO™ water (v/v) at a ratio of 1:10, 1:100, 1:1000 and 1:10000 to test for inhibition of the qPCR reaction.

3 RESULTS

3.1 Potted plant trial

3.1.1 Baseline

There were leaf spot symptoms on the 'Hort16A' plants before treatments were applied. There was evidence that 'Hort16A' was infected with Psa-LV, but probably not Psa-V from one of the two samples taken immediately before treatment. This sample had a Ct value of 21.75 with the F3/R4 primers, but was not positive with the HopZ2b primers. One sample of G14 had a Ct value of 29.6 with the F3/R4 primers, but was not positive with HopZ2b primers, so may also have been infected with Psa-LV but not Psa-V. All other samples were negative.

3.1.2 Lesion length

Lesion lengths did not show any obvious pattern according to treatment. For instance, the inoculated control would be expected to show the most extensive lesions, but did not for G14 or G11 (Table 5 and 7). For 'Hort16A', inoculations of untreated wounds at 10^6 cfu/mL resulted in lesions that were longer than all other treatments (Table 6).

Only for G11 were there any statistically significant differences between treatments (Table 7). However, no wound treatment resulted in a significant reduction in lesion length compared with the inoculated control.

3.1.3 Ct values

1. Ct values < 30

    a. HopZ2b primers

If the number of Ct values that were less than 30 are considered to be negative for Psa-V and are compared, then for G11, InocBloc paste was the best treatment, and application resulted in a statistically significant reduction in the number of infected canes (Figure 3, Table 2). The treatment resulting in the most infection by Psa-V was the inoculated control.

For G14, the treatment resulting in the most infection by Psa-V was Prunesafe, and the best treatment was copper paste, although the reduction in incidence was not significant when compared with inoculated controls (Table 3, Figure 4).

For 'Hort16A', the treatment resulting in the most infection by Psa-V was Prunesafe, followed by Product A and the inoculated controls, and the best treatment was copper paste, followed by Vinevax and Actigard (Table 4, Figure 5). However, none of these treatments were significantly different to the inoculated controls.

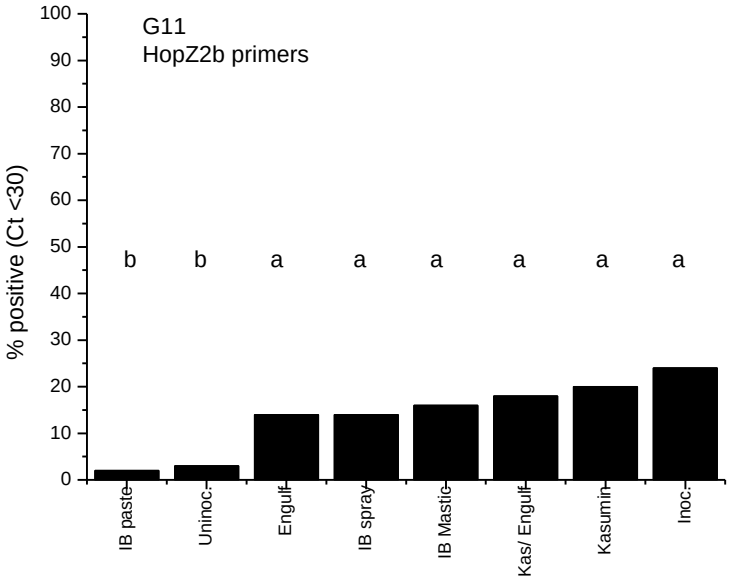


Figure 3. Untransformed incidence data for numbers of *Actinidia chinensis var. deliciosa* 'Zes007' G11 kiwifruit canes infected with Psa-V (Ct<30; n = 30) following wounding, application of protectants, inoculation with Psa-V and assessment 11 weeks later. Values followed by the same letters are not significantly different according to Tukey's test at α = 0.05 following statistical analysis of arcsine transformed data. Treatments are IB = InocBloc, uninoc.= uninoculated controls, Kas/Engulf = Kasumin + Engulf, Inoc = inoculated controls. (10 potted vines per treatment).

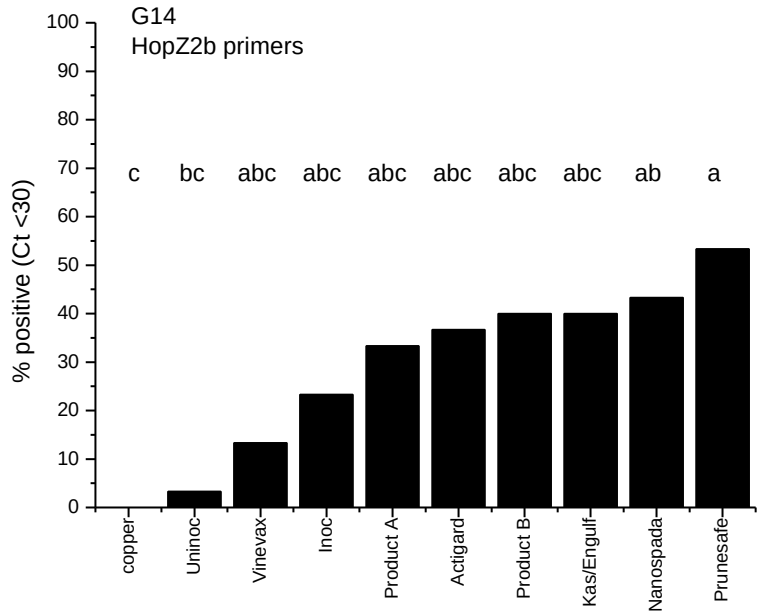


Figure 4. Untransformed incidence data for numbers of *Actinidia chinensis* var. *chinensis* x *A. chinensis* var. *deliciosa* 'Zesh004' G14 kiwifruit canes infected with Psa-V (Ct<30; n = 30) following wounding, application of protectants, inoculation with Psa-V and assessment 11 weeks later. Values followed by the same letters are not significantly different according to Tukey's test at α = 0.05 following statistical analysis of arcsine transformed data. Treatments are uninoc.= uninoculated controls, Kas/Engulf = Kasumin + Engulf, Inoc = inoculated controls, copper = copper paste. (10 potted vines per treatment).

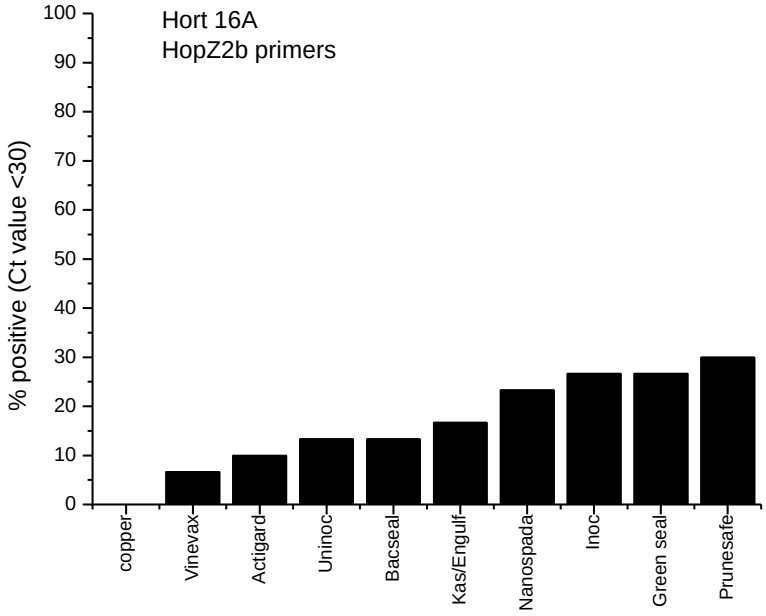


Figure 5. Untransformed incidence data for numbers of *Actinidia chinensis* var. *chinensis* 'Hort16A' kiwifruit canes infected with Psa-V (Ct<30; n = 30) following wounding, application of protectants, inoculation with Psa-V and assessment 11 weeks later. Treatments are uninoc.= uninoculated controls, Kas/Engulf = Kasumin + Engulf, Inoc = inoculated controls, copper = copper paste. (10 potted vines per treatment).

b. F3/R4 primers

The results with the F3/R4 primers were almost the same as for the HopZ2b primers, and graphs are presented in Appendix 2. The best treatments, resulting in the least infected canes were, for G11 InocBloc paste (Table 2), for G14 (Table 3) copper paste and for 'Hort16A' copper paste and Actigard (Table 4).

The treatments with the highest number of infected canes were, for G11 the inoculated controls, G14 Prunesafe, and for 'Hort16A' Product A>Prunesafe>inoculated controls.

Table 2. Back transformed incidence (Ct < 30) data from qPCR reactions with F3/R4 and HopZ2b primers for *Actinidia chinensis* var. *deliciosa* 'Zes007' G11 kiwifruit canes sampled 11 weeks after wounding, application of wound protectants and inoculation with Psa-V. Values were arcsine transformed before analysis. (n= 10 potted vines per treatment, and three samples per vine).

	F3/R4 primers		HopZ2b primers	
Product	**%**	**Tukey's**	**%**	**Tukey's**
1. Engulf™	79.9	ab	43.9	a
2. InocBloc™ paste	23.4	c	0.91	b
3. InocBloc mastic	74.8	abc	56.1	a
4. InocBloc spray	79.4	ab	43.9	a
5. Kasumin	96.6	ab	76.6	a
6. Kasumin/Engulf	97.6	a	65.5	a
7. Inoculated	88.8	ab	90.5	a
8. Uninoculated	56.1	bc	2.4	b
Statistical analysis: ANOVA P values				
Treatment	<0.0001		<0.0001	
Vine	0.081		0.032	

Values followed by the same letters are not significantly different according to Tukey's test at α = 0.05.

Table 3. Back transformed incidence (Ct < 30) data from qPCR reactions with F3/R4 and HopZ2b primers for *Actinidia chinensis* var. *chinensis* x *A. chinensis* var. *deliciosa* 'Zesh004' G14 kiwifruit canes sampled 11 weeks after wounding, application of wound protectants and inoculation with Psa-V. Values were arcsine transformed before analysis. (n= 10 potted vines per treatment and three samples per vine).

	F3/R4 primers		HopZ2b primers	
Product	**%**	**Tukey's**	**%**	**Tukey's**
9. Actigard™	59.5	ab	31.4	abc
10. Product B	59.5	ab	34.5	abc
11. copper paste	0	c	0	c
12. Product A	56.1	ab	25.8	abc
13. Kasumin/ Engulf	68.6	ab	37.2	abc
14. Nanospada™	68.6	ab	40.5	ab
15. Prunesafe	86.5	a	50.6	a
16. Vinevax™	40.5	abc	4.7	abc
17. Inoculated	25.2	abc	13.5	abc
18. Uninoculated	13.5	bc	0.4	bc
Statistical analysis: ANOVA P values				
Treatment	<0.0001		0.001	
Vine	0.516		0.959	

Values followed by the same letters are not significantly different according to Tukey's test at α = 0.05.

Table 4. Back transformed incidence (Ct < 30) data from qPCR reactions with F3/R4 and HopZ2b primers for *Actinidia chinensis* var. *chinensis* 'Hort16A' kiwifruit canes sampled 11 weeks after wounding, application of wound protectants and inoculation with Psa-V. Values were arcsine transformed before analysis. (n= 10 potted vines per treatment and three samples per vine).

	F3/R4 primers	HopZ2b primers
Product	**%**	**%**
1. Actigard™	67.4	3.4
2. Product B	82.9	2.4
3. copper paste	52.6	0.4
4. Product A	92.0	7.7
5. Kasumin/Engulf	80.1	7.7
6. Nanospada™	80.1	13.5
7. Prunesafe	88.1	11.2
8. Vinevax™	75.2	0.4
9. Inoculated	86.6	11.2
10. Uninoculated	81.4	0.9
Statistical analysis: ANOVA P values		
Treatment	0.133	0.361
Vine	0.043	0.932

2. Mean Ct values

For all three kiwifruit varieties, the untreated wounds resulted in the lowest Ct values of canes inoculated with 10^4 cfu/mL (Tables 5, 6 and 7). For plants inoculated with 10^6 cfu/mL, the inoculated untreated wounds did not result in the lowest Ct values.

There were no significant differences in Ct values for plants inoculated with differing amounts of inoculum, but there was a significant interaction of treatment and inoculum for G14 and G11 (Tables 5 and 7).

There were statistically significant differences in Ct values between treatments for G14, G11 and 'Hort16A', for both HopZ2b and F3/R4 primers (Tables 5, 6 and 7).

For G14 (Table 5), wounds treated with copper paste, and for G11 (Table 7) wounds treated with InocBloc paste had significantly higher Ct values than the inoculated controls. For 'Hort16A' the treatment that resulted in the highest Ct value was copper paste. There was a significant difference to inoculated controls when the F3/R4 primers were used (Table 6).

No other wound protectants showed significantly higher Ct values when compared with the inoculated controls.

Application of Prunesafe to wounds of G14 vines resulted in a significantly lower Ct value than inoculated controls (Table 5).

Ct values of samples taken at the cut end, 5 and 10 cm below the wound were significantly different for G14 and ‘Hort16A’, but not for G11. Ct values of the samples from the cut end were significantly lower than all other samples for G14, and Ct values of samples from 10 cm below the wound were significantly higher than all other samples for ‘Hort16A’ (Appendix 1).

Table 5. Lesion lengths and Ct values from qPCR reactions with F3/R4 and HopZ2b primers for *Actinidia chinensis* var. *chinensis* x *A. chinensis* var. *deliciosa* 'Zesh004' G14 kiwifruit canes sampled 11 weeks after wounding, application of wound protectants and inoculation with two concentrations of Psa-V. (n= 10 potted vines per treatment and three samples per vine).

Treatment	Lesion length (mm)		Tukey's	F3/R4 primers		Tukey's	HopZ2b primers		Tukey's
	Inoculum (cfu/mL)								
	10^4	10^6		10^4	10^6		10^4	10^6	
1. copper paste	2.9±1.13	4.3±2.2	n.s.	38.5±0.75	39.3±0.53	a	40±0	40±0	a
2. Product B	2.4±2.4	0.8±0.34	n.s.	28.7±2.28	26.2±2.79	bcd	34.6±1.71	32.6±2.15	bcd
3. Product A	15.6±14.9	1.3±0.72	n.s.	29.2±2.15	23.9±2.60	cd	35.8±1.90	32.0±2.25	bcd
4. Vinevax™	0.6±0.30	1.1±0.51	n.s.	33.4±1.55	29.9±2.23	bc	38.3±0.74	35.4±1.66	ab
5. Actigard™	22.9±21.5	33.7±19.1	n.s.	25.1±2.16	29.9±1.96	bcd	31.3±1.72	35.4±1.47	bcd
6. Nanospada™	203±154	10.5±9.38	n.s.	28.9±2.25	23.8±1.94	cd	35.1±1.54	30.1±1.88	bcd
7. Prunesafe	8.5±5.32	2.1±1.47	n.s.	25.8±2.55	21.9±1.82	d	30.4±2.18	28.6±2.13	d
8. Kasumin/Engulf	161±161	1.6±0.75	n.s.	28.6±1.89	25.8±2.31	bcd	33.3±1.48	29.7±1.91	cd
9. inoculated control	11.2±9.72	1.7±1.36	n.s.	27.9±2.45	36.0±1.21	bc	30.7±2.05	38.7±0.63	bc
10. uninoculated control	158±157	8.4±8.15	n.s.	33.1±1.23	33.7±1.09	ab	37.3±0.73	38.17±0.63	ab
				Statistical analysis: ANOVA P-values					
Treatment	0.531			<0.0001			<0.0001		
Depth	n.a.			0.004			0.001		
Inoculum	0.062			0.306			0.459		
Treatment*inoculum	0.567			0.007			0.001		

n.s. = not significant; n.a. = not applicable. Values followed by the same letters are not significantly different according to Tukey's test at α = 0.05.

Table 6. Lesion lengths and Ct values from qPCR reactions with F3/R4 and HopZ2b primers for *Actinidia chinensis* var. *chinensis* 'Hort16A' kiwifruit canes sampled 11 weeks after wounding, application of wound protectants and inoculation with two concentrations of Psa-V. (n= 10 potted vines per treatment and three samples per vine).

Treatment	Lesion length (mm)		Tukey's	F3/R4 primers		Tukey's	HopZ2b primers		Tukey's
				Inoculum (cfu/mL)					
	10^4	10^6		10^4	10^6		10^4	10^6	
1. copper paste	120±101.3	6.2±0.66	n.s.	35.2±1.86	37.5±1.34	a	38.7±1.09	39.4±0.44	a
2. Product B	354.2±144.93	307.8±121.02	n.s.	30.7±2.04	30.0±2.62	abc	38.1±0.71	36.28±1.63	ab
3. Product A	268.0±152.24	135.1±118.88	n.s.	28.5±2.32	25.3±2.00	c	37.78±0.85	33.9±1.52	ab
4. Vinevax™	144.0±129.52	108.8±100.42	n.s.	31.6±2.18	33.9±1.74	abc	37.5±1.24	39.4±0.37	ab
5. Actigard™	37.2±12.68	19.0±15.28	n.s.	31.5±2.45	35.9±1.77	ab	37.90±1.24	38.6±0.98	ab
6. Nanospada™	213.7±131.59	256.3±154.82	n.s.	29.2±2.22	33.2±2.40	abc	35.02±1.40	36.4±1.49	ab
7. Prunesafe	134.3±126.49	197.4±94.40	n.s.	30.8±2.39	27.8±2.10	bc	35.7±1.57	34.0±1.40	b
8. Kasumin/Engulf	287.6±117.07	4.8±4.8	n.s.	28.7±2.28	31.7±1.47	abc	34.6±1.38	39.1±0.88	ab
9. inoculated control	238.1±138.17	544.0±144.73	n.s.	28.2±2.20	31.5±2.17	bc	34.4±1.42	37.0±1.43	ab
10. uninoculated control	135.5±123.90	140.0±140.0	n.s.	28.4±2.03	33.9±1.41	abc	36.2±1.55	38.7±0.65	ab
				Statistical analysis: ANOVA P-values					
Treatment	0.064			0.001			0.006		
Depth	n.a.			<0.0001			<0.0001		
Inoculum	0.680			0.046			0.199		
Treatment*inoculum	0.550			0.329			0.023		

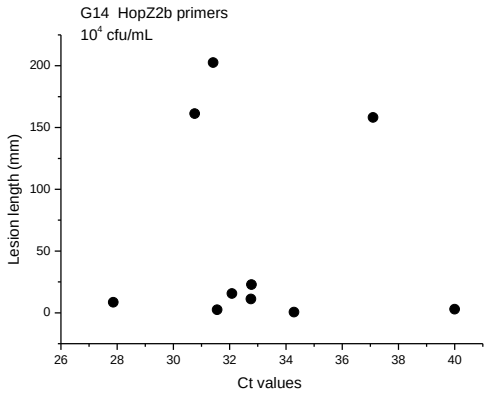
n.s. = not significant; n.a. = not applicable. Values followed by the same letters are not significantly different according to Tukey's test at α = 0.05.

Table 7. Lesion lengths and Ct values from qPCR reactions with F3/R4 and HopZ2b primers for *Actinidia chinensis* var. *deliciosa* 'Zes007' G11 kiwifruit canes sampled 11 weeks after wounding, application of wound protectants and inoculation with two concentrations of Psa-V. (n= 10 potted vines per treatment and three samples per vine).

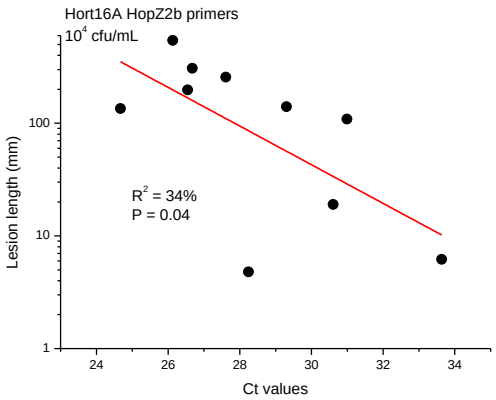
Treatment	Lesion length (mm)		Tukey's	F3/R4 primers		Tukey's	HopZ2b primers		Tukey's
				Inoculum (cfu/mL)					
	10^4	10^6		10^4	10^6		10^4	10^6	
1. InocBloc™ spray	8.2±4.56	6.2±1.02	a	25.3±1.97	26.4±2.43	bc	29.2±1.78	25.9±1.30	bc
2. InocBloc paste	6.4±2.55	3.1±0.89	ab	32.5±2.15	34.7±1.45	a	34.63±2.50	34.9±0.83	a
3. InocBloc mastic	1.6±0.53	3.8±1.24	ab	27.1±1.75	24.0±2.52	bc	31.6±1.45	25.25±1.46	c
4. Kasumin	0.9±0.68	1.5±0.42	b	23.1±2.02	20.5±1.45	c	27.4±1.77	26.1±1.46	c
5. Kasumin/Engulf	0.6±0.40	3.0±1.36	b	24.7±2.02	22.0±1.36	c	29.0±1.83	27.5±1.44	c
6. Engulf™	2.6±1.13	3.4±2.19	ab	25.1±1.42	26.4±1.88	bc	31.2±1.47	29.3±1.29	bc
7. inoculated control	1.8±0.56	3.2±0.93	ab	23.1±2.11	23.2±1.99	c	25.7±1.28	26.6±1.01	c
8. uninoculated control	1.5±0.82	1.5±0.39	b	29.4±1.48	31.2±0.95	ab	34.0±1.25	35.8±0.78	ab
				Statistical analysis: ANOVA P-values					
Treatment	0.008			<0.0001			<0.0001		
Depth	n.a.			0.721			0.764		
Inoculum	0.743			0.790			0.223		
Treatment*inoculum	0.616			0.662			0.471		

Values followed by the same letters are not significantly different according to Tukey's test at α = 0.05.

a)



b)



c)

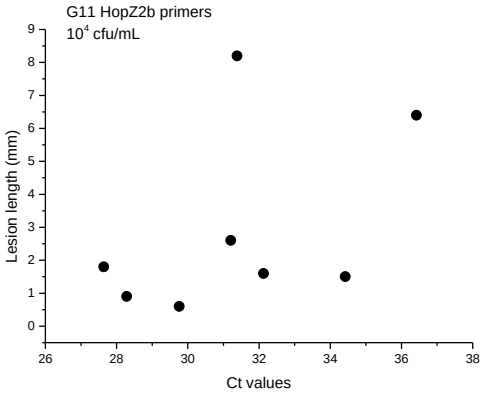


Figure 6. Lesion lengths plotted against Ct values from qPCR reactions with HopZ2b primers for a) *Actinidia chinensis* var. *chinensis* x *A. chinensis* var. *deliciosa* 'Zesh004' G14, b) *A. chinensis* var. *chinensis* 'Hort16A' and c) *A. chinensis* var. *deliciosa* 'Zes007' G11 kiwifruit canes sampled 11 weeks after wounding, application of wound protectants and inoculation with Psa-V. Potted plants were spray inoculated with either 10^4 cfu/mL Psa-V immediately after wounding and application of wound protectants.

3.2 Mature vine trials

3.2.1 Winter pruning

Baseline levels

There were no statistically significant differences between treatments or vines when the samples taken immediately before application of wound treatments were tested with the HopZ2b primers in qPCR. Of 540 canes that were tested, there were 11 (2.0%) samples with a Ct value <30. With the F3/R4 primers, there was a treatment effect (*P*=0.043) but the treatments were not able to be separated using Tukey's test. There was no significant difference between vines, and 19 (3.5%) canes with a Ct value <30 (Appendix 4).

After 2 weeks

There was a significant treatment effect and a significant difference between vines for incidence and for Ct values (Table 8 and 9). Vines 6, 2, 8 and 4 were significantly more infected by Psa-V than were Vines 10, 5 and 11 (Appendix 6).

There was a significant treatment and a significant vine effect for both Ct values and incidence (Table 8 and 9). With both sets of primers, Ct values for canes treated with copper paste or InocBloc paste and unwounded controls were significantly lower than Ct values for wounded untreated controls (Table 9). A significantly fewer number of canes treated with copper or InocBloc were infected with Psa-V (Ct values <30) than untreated wounded controls (Figure 7; Table 8) when tested with the HopZ2b primers.

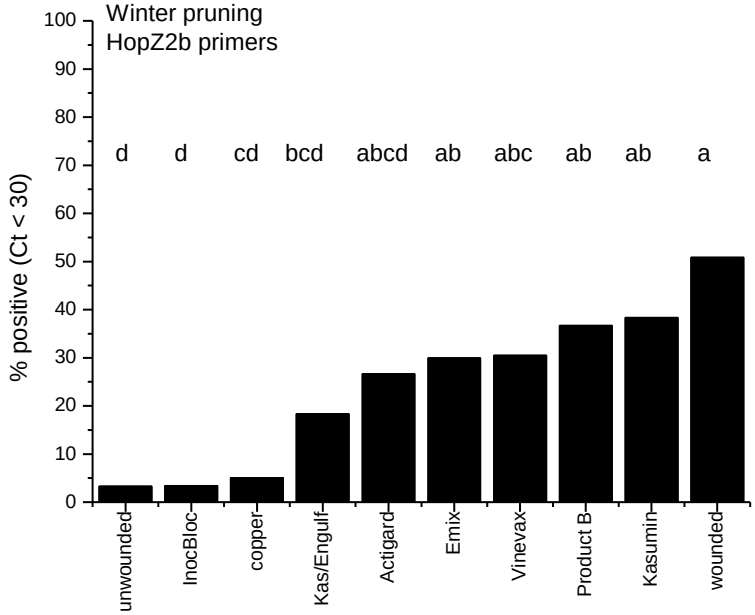


Figure 7. Untransformed incidence data for numbers of *Actinidia deliciosa* 'Chieftain' kiwifruit canes naturally infected with Psa-V (Ct<30) following wounding in winter, application of protectants, and assessment 2 weeks later. Values followed by the same letters are not significantly different according to Tukey's test at α = 0.05 following statistical analysis of arcsine transformed data. Treatments are unwounded = unwounded untreated controls, Kas/Engulf = Kasumin + Engulf®, wounded = wounded untreated controls, copper = copper paste. (n= 60 per treatment, 12 vines and 5 canes per vine).

Table 8. Back transformed incidence (n = 540) values (Ct<30) from qPCR reactions with F3/R4 and HopZ2b primers for *Actinidia deliciosa* 'Chieftain' kiwifruit canes sampled 2 weeks after wounding in winter, application of wound protectants and naturally infected with Psa-V. Data were arcsine transformed before analysis. (n= 60 per treatment, 12 vines and 5 canes per vine).

	F3/R4 primers		HopZ2b primers	
Product	%	Tukey's	%	Tukey's
Actigard™	56.7	a	19.2	abcd
Product B	53.5	a	32.1	ab
Copper	15.1	ab	0.91	cd
Emix	50.2	a	27.2	ab
InocBloc™ paste	23.7	ab	0.60	d
Kasumin	39.2	ab	33.5	ab
Kasumin/Engulf®	42.8	ab	11.3	bcd
Vinevax™	59.3	a	23.7	abc
wounded	49.3	a	52.2	a
unwounded	6.7	b	0.6	d
Statistical analysis: ANOVA P values				
Treatment	<0.0001		<0.0001	
Vine	<0.0001		<0.0001	

Values followed by the same letters are not significantly different according to Tukey's test at α = 0.05.

Table 9. Ct values from qPCR reactions with F3/R4 and HopZ2b primers for *Actinidia deliciosa* 'Chieftain' kiwifruit canes sampled 2 weeks after wounding in winter, application of wound protectants and naturally infected with Psa-V. (n= 60 per treatment, 12 vines and 5 canes per vine).

Treatment	F3/R4 primers	Tukey's	HopZ2b	Tukey'
Actigard™	27.8 ±1.25	c	34.6±0.90	b
Product B	28.8 ±1.32	c	32.7±1.08	bc
Copper paste	34.8 ±0.94	ab	38.9±0.44	a
Emix	30.2 ±1.26	bc	33.9±0.93	bc
InocBloc™ paste	34.3 ±1.13	ab	39.5±0.28	a
Kasumin	31.2 ±1.23	bc	32.8±1.02	bc
Kasumin/Engulf	30.3 ±1.18	bc	35.5±0.85	b
Vinevax™	29.5 ±1.28	c	33.9±0.98	bc
Unwounded	36.6 ±0.86	a	38.8±0.45	a
Wounded	28.3 ±1.33	c	31.0±1.03	c
Statistical analysis: ANOVA P-values				
Treatment	<0.0001		<0.0001	
Vine	<0.0001		<0.0001	
Treatment*Vine	<0.0001		<0.0001	

Values followed by the same letters are not significantly different according to Tukey's test at α = 0.05.

3.2.2 Spring pruning

Baseline

There were no statistically significant differences between treatments when the samples were taken immediately before wounding and application of protectants when tested with both F3/R4 and HopZ2b primers. There were statistically significant differences between vines, with the Ct values for Vine 3 being significantly lower than those of Vines 1, 2 and 6 when both primers were used. Of 300 canes that were sampled, 28 (9.3%) had a Ct value <30 after testing with F3/R4 primers, and 13 (4.3%) after testing with HopZ2b primers (Appendix 7).

After 2 weeks

There was a significant treatment and a significant vine effect. There were significantly lower Ct values for Vines 2 and 3 compared with Vines 1 and 5 when both primer sets (F3/R4 and HopZ2b) were used (Appendix 9).

A significantly fewer number of canes treated with copper or InocBloc were infected with Psa-V (Ct values <30) than untreated wounded controls (Table 10, Figure 8) when tested with both sets of primers (F3/R4 and HopZ2b). Ct values for canes treated with copper or InocBloc paste and unwounded controls were significantly higher than Ct values for wounded untreated controls (Table 11).

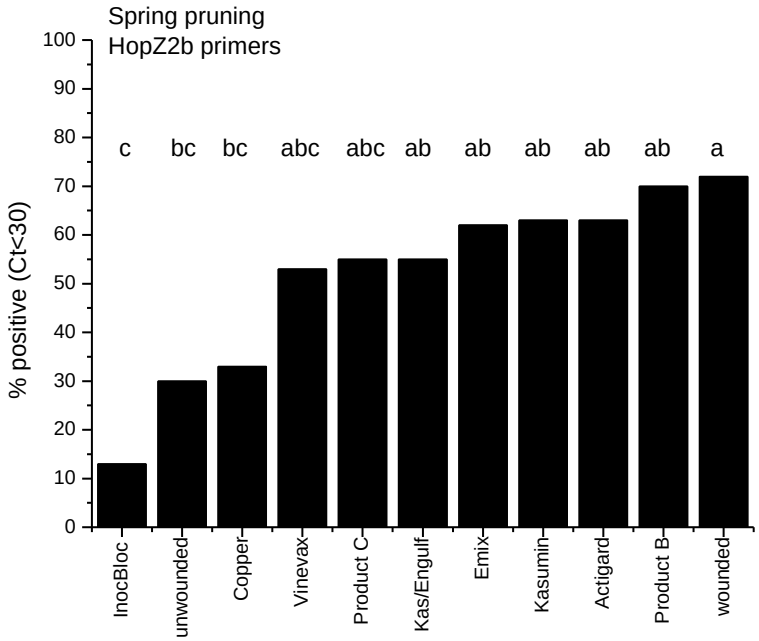


Figure 8. Untransformed incidence data for numbers of *Actinidia deliciosa* 'Chieftain' kiwifruit canes naturally infected with Psa-V (Ct<30) following wounding in spring, application of protectants, and assessment 2 weeks later. Values followed by the same letters are not significantly different according to Tukey's test at α = 0.05 following statistical analysis of arcsine transformed data. Treatments are unwounded = unwounded untreated controls, Kas/Engulf = Kasumin + Engulf®, wounded = wounded untreated controls, copper = copper paste. (n = 30 per treatment, 6 vines and 5 canes per vine).

Table 10. Back transformed incidence (n = 540) values (Ct<30) from qPCR reactions with F3/R4 and HopZ2b primers for *Actinidia deliciosa* 'Chieftain' kiwifruit canes sampled 2 weeks after wounding in spring, application of wound protectants and naturally infected with Psa-V. Data were arcsine transformed before analysis. (n= 30 per treatment, 6 vines and 5 canes per vine).

	Chieftain spring pruning			
	F3/R4 primers		HopZ2b primers	
Product	%	Tukey's	%	Tukey's
Actigard™	98.7	a	83.6	ab
Product B	97.6	a	85.8	ab
Copper	49.0	bcd	32.1	bc
Emix	88.5	abc	77.6	ab
InocBloc™ paste	19.2	d	8.9	c
Kasumin	96.4	ab	86.5	ab
Kasumin/Engulf®	84.1	abc	75.0	ab
Product C	74.4	abcd	67.4	abc
Vinevax™	88.9	abc	67.7	abc
wounded	96.4	a	94.9	a
unwounded	46.0	cd	28.0	bc
Statistical analysis: ANOVA P values				
Treatment	<0.0001		<0.0001	
Vine	0.002		<0.0001	

Values followed by the same letters are not significantly different according to Tukey's test at α = 0.05.

Table 11. Ct values from qPCR reactions with F3/R4 and HopZ2b primers for *Actinidia deliciosa* 'Chieftain' kiwifruit canes sampled 2 weeks after wounding in spring, application of wound protectants and naturally infected with Psa-V. (n= 30 per treatment, 6 vines and 5 canes per vine).

Treatment	F3/R4 primers	Tukey's	HopZ2b primers	Tukey's
1. Actigard™	19.7±1.12	c	25.1±1.09	b
2. Product B	19.7±1.26	c	24.3±1.03	b
3. Copper paste	29.7±1.43	a	33.6±1.14	a
4. Emix	21.0±1.59	c	25.4±1.32	b
5. InocBloc™ paste	34.2±1.68	a	36.1±1.29	a
6. Kasumin	19.7±1.26	c	25.3±1.24	b
7. Kasumin/Engulf	22.3±1.76	c	27.3±1.60	b
8. Vinevax™	22.5±1.61	c	27.5±1.39	b
9. Product C	23.2±1.93	bc	27.4±1.62	b
Unwounded	28.9±1.78	ab	32.8±1.46	a
Wounded	18.7±1.23	c	23.5±1.10	b
Statistical analysis : ANOVA P-values				
Treatment	<0.0001		<0.0001	
Vine	<0.0001		<0.0001	
Treatment*Vine	<0.0001		<0.0001	

Values followed by the same letters are not significantly different according to Tukey's test at α = 0.05.

3.2.3 Dilution series with InocBloc samples

Psa was not detected with either the F3/R4 or the HopZ2b primers when the InocBloc samples were diluted stepwise to 1:10,000 v/v.

4 DISCUSSION

InocBloc paste and copper paste consistently protected pruning wounds on kiwifruit vines from infection by Psa-V, in both potted plant trials and in the field.

Analysis of Ct values showed more statistically significant differences than incidence data, as would be expected because there was more replication, and there is more information available from qualitative data (Ct values) compared with quantitative (incidence) data.

The length of lesions was completely unrelated to the amount of Psa that was detected in canes for G11 and G14, and was not a reliable indicator of the effectiveness of wound protectants for 'Hort16A'. By qPCR the inoculated or wounded controls were almost always the worst treatments, and the uninoculated or unwounded controls the best treatments, but this was not the case for lesion length. Production of the phenolics that are responsible for the brown staining observed in infected plants is a host defence response, and in some instances probably effectively prevented colonisation by Psa. qPCR detects the bacterial cells rather than the host response, and is thus a more accurate assessment of infection by Psa.

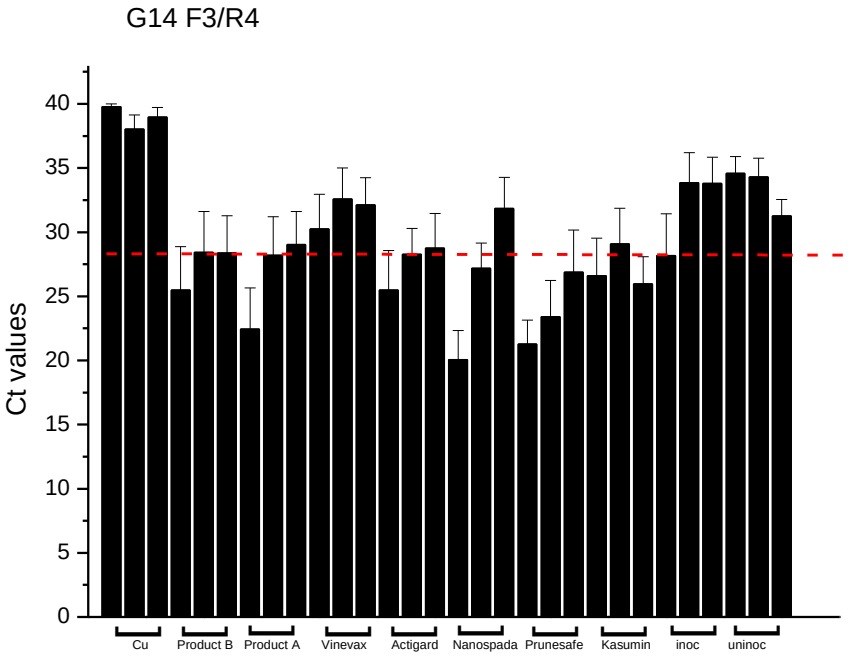
For potted plants, copper paste provided statistically significant wound protection compared with inoculated controls for G14 and 'Hort16A' when mean Ct values were analysed. It is apparent from the count data (% canes infected) that copper paste was the best wound protectant although this was not statistically significant.

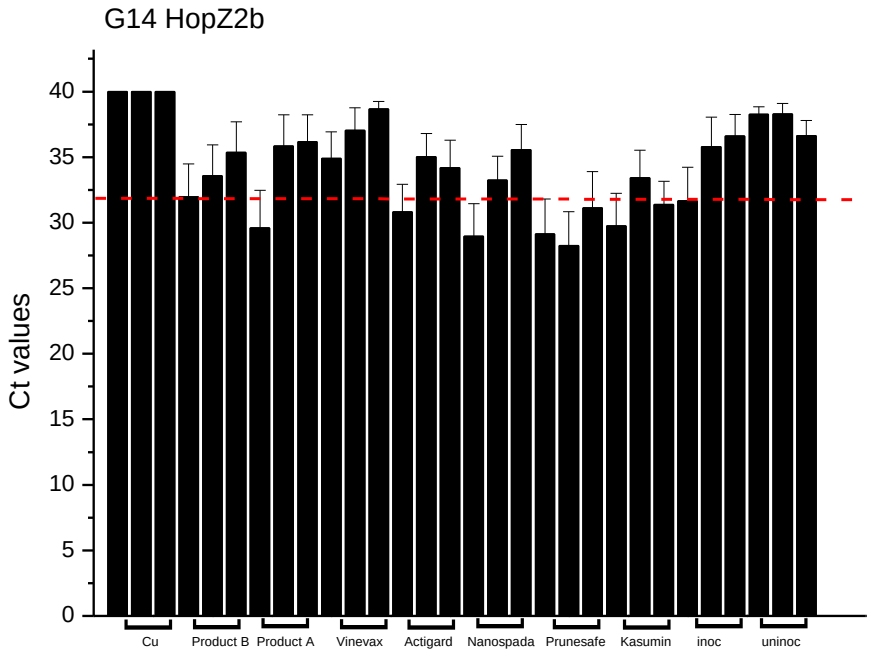
On G11 potted plants, InocBloc paste provided statistically significant wound protection compared with untreated controls for both incidence data and mean Ct values.

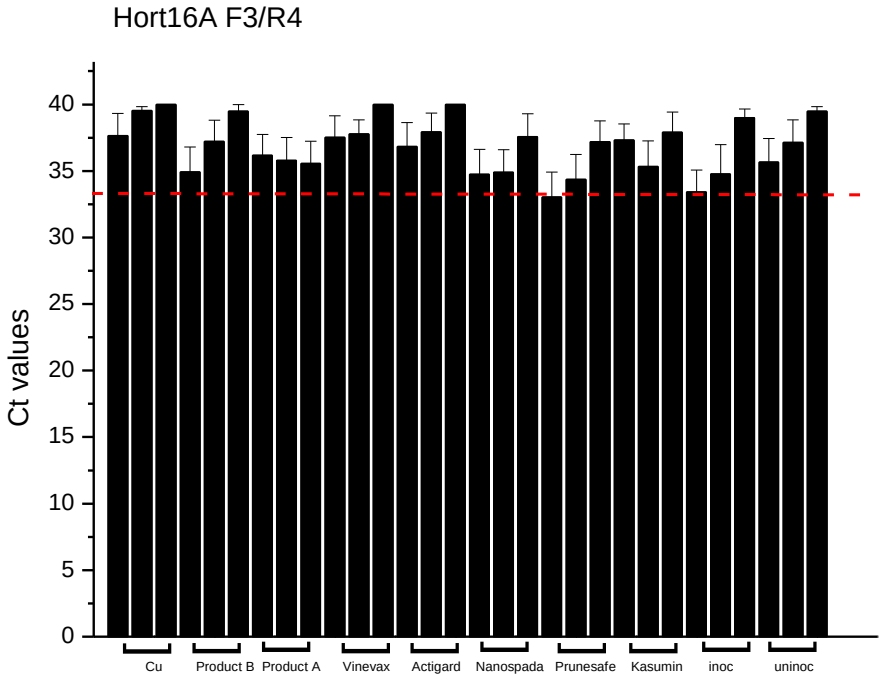
On 'Chieftain' male kiwifruit vines in the field, copper paste and InocBloc paste provided statistically significant wound protection compared with controls when applied both in winter and spring, both from incidence data and from Ct values. InocBloc paste appeared to provide better protection than copper paste, although this was not statistically significant.

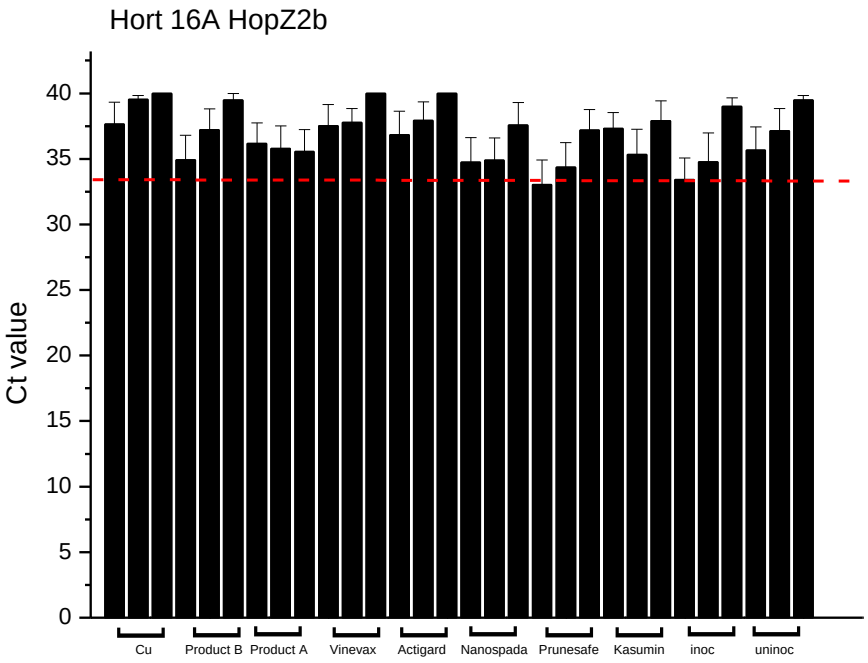
Without application of wound protectants, the incidence of 'Chieftain' kiwifruit canes naturally infected by Psa in winter increased from 3.3% to 50% in the 2 weeks after wounding, and in spring from 30% to 72%. It would be advisable to apply an effective wound protectant such as copper paste or InocBloc paste immediately after pruning to reduce infection of vines in the field.

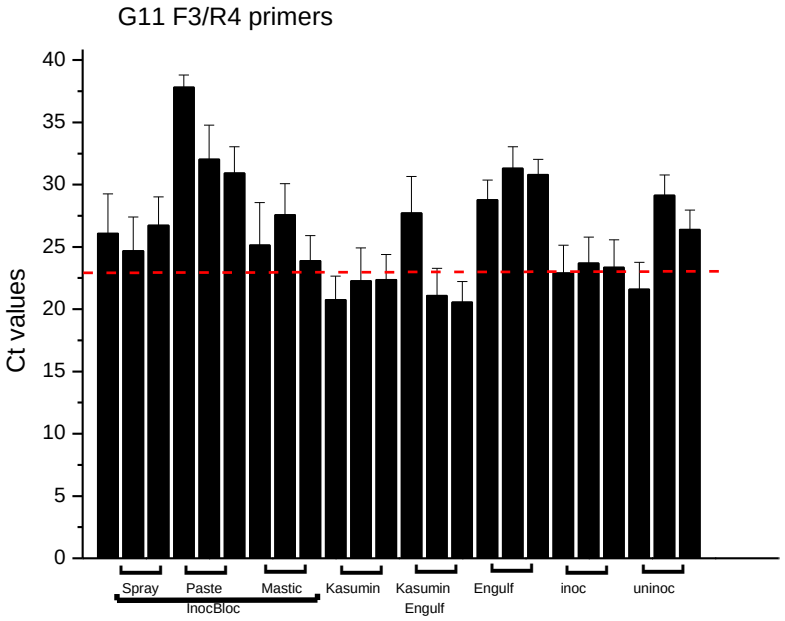
APPENDICES

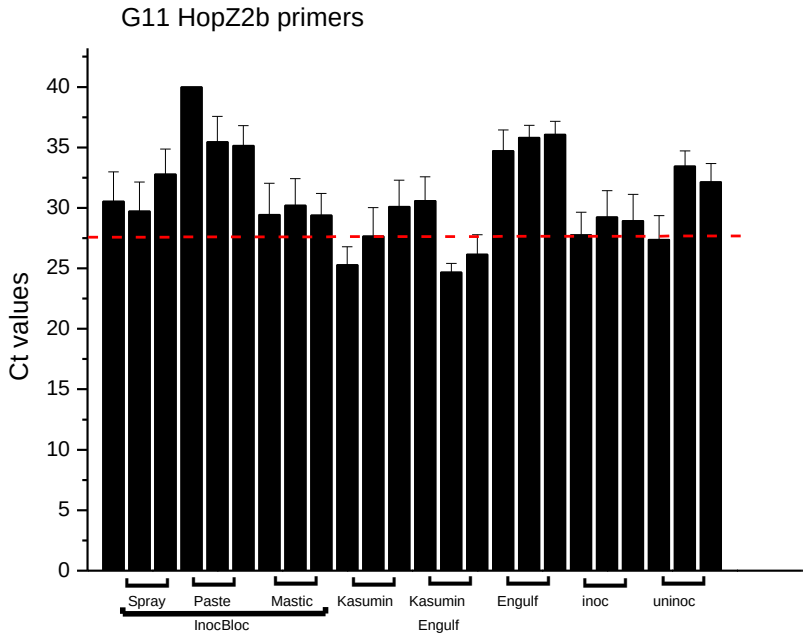




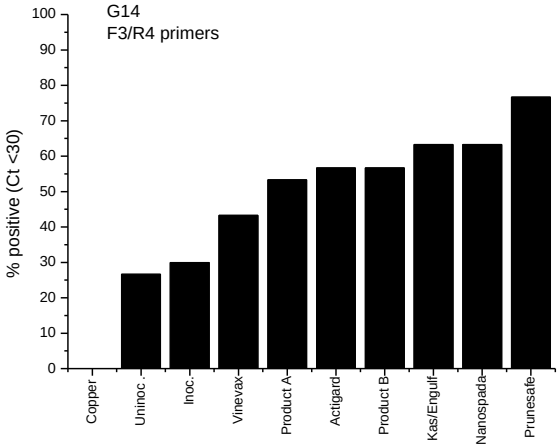


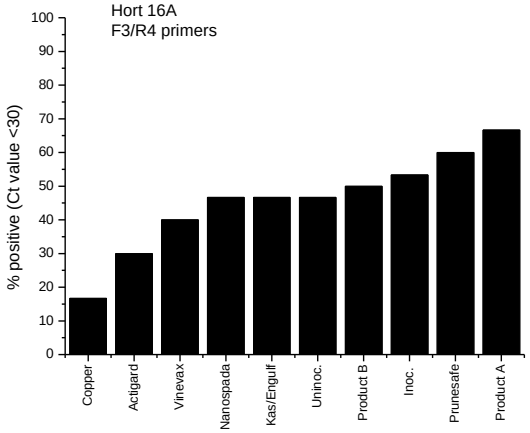


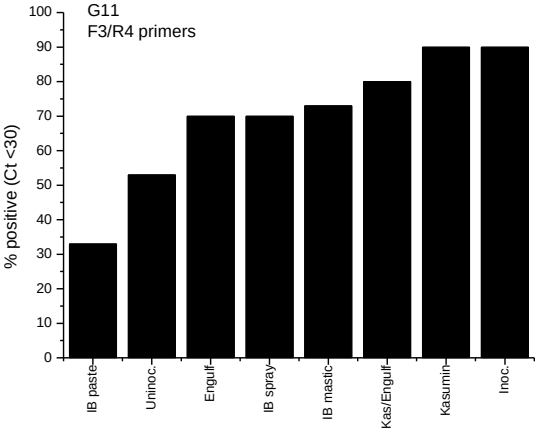




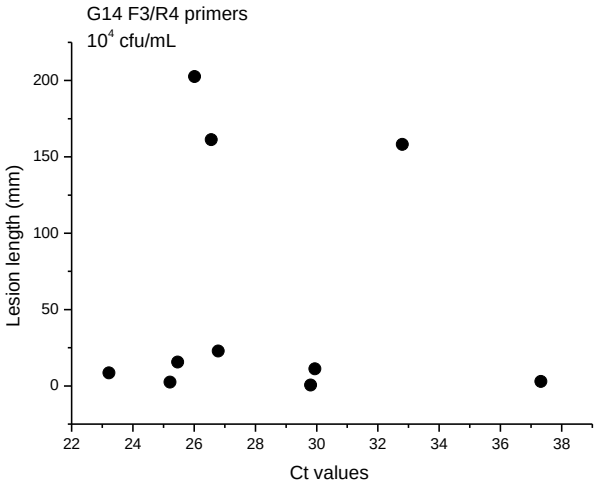
Appendix 1. Ct values (mean ± standard error) at the cut end, 5 cm below the wound and 10 cm below the wound (left to right) for 10 potted vines per treatment of kiwifruit varieties *Actinidia chinensis* var. *deliciosa* 'Zes007' G11, *A. chinensis* var. *chinensis* x *A. chinensis* var. *deliciosa* 'Zesh004' G14, *A. chinensis* var. *chinensis* 'Hort16A'tested with primers F3/R4 and HopZ2b in qPCR reactions following wounding, application of protectants, inoculation with Psa-V and assessment 11 weeks later. Dotted red line denotes the Ct value for the wounded inoculated control treated canes at the cut end.

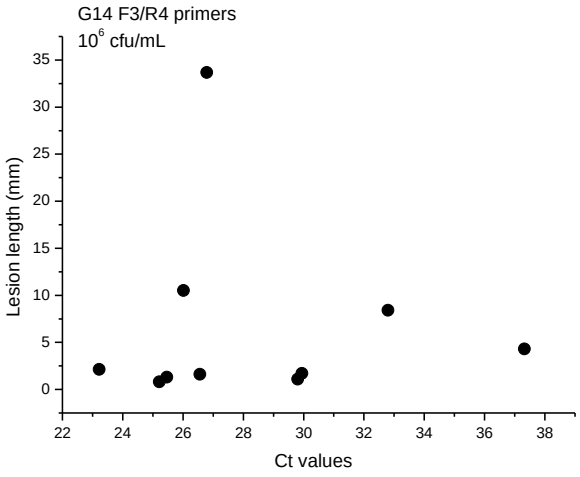


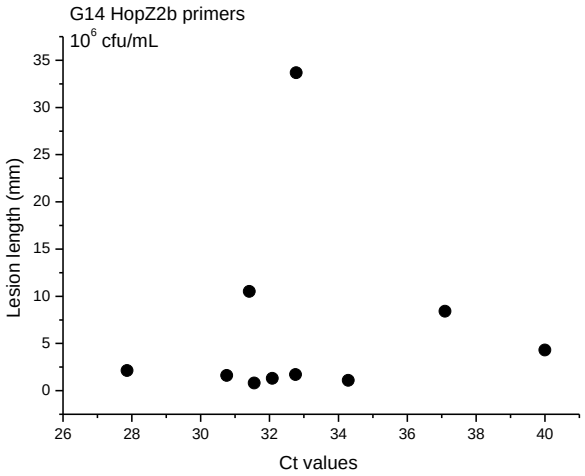


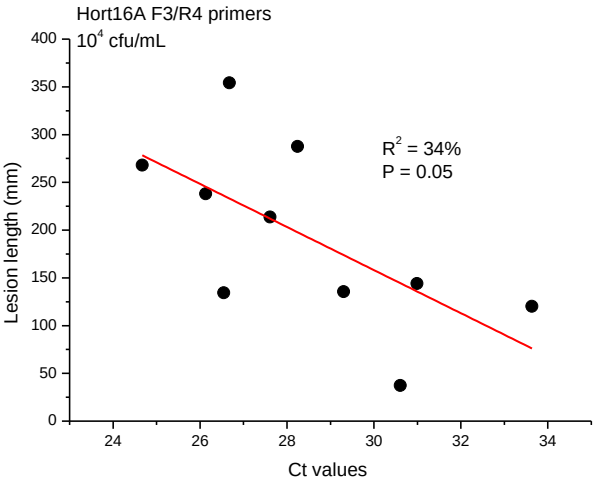


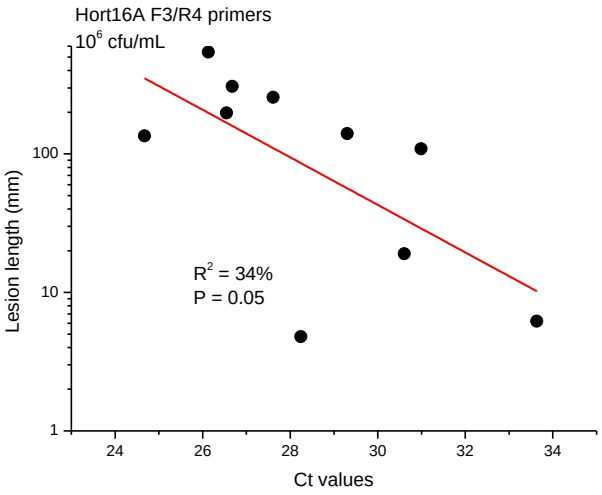
Appendix 2. Untransformed incidence values for kiwifruit varieties *Actinidia chinensis* var. *deliciosa* 'Zes007' G11, *A. chinensis* var. *chinensis* x *A. chinensis* var. *deliciosa* 'Zesh004' G14, *A. chinensis* var. *chinensis* 'Hort16A'tested with primers F3/R4 and HopZ2b in qPCR reactions following wounding, application of protectants, inoculation with Psa-V and assessment 11 weeks later. Values are incidence (%) of 10 canes sampled at the cut end, 5 and 10 cm below the wound (n = 30). Treatments are IB = InocBloc, uninoc.= uninoculated controls, Kas/Engulf = Kasumin + Engulf®, Inoc = inoculated controls, copper = copper paste.

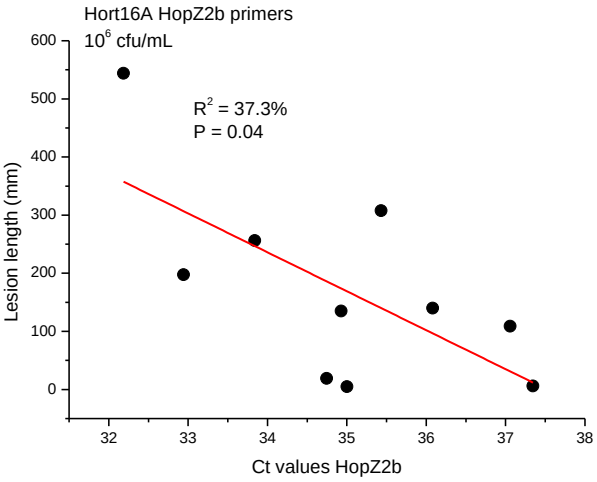


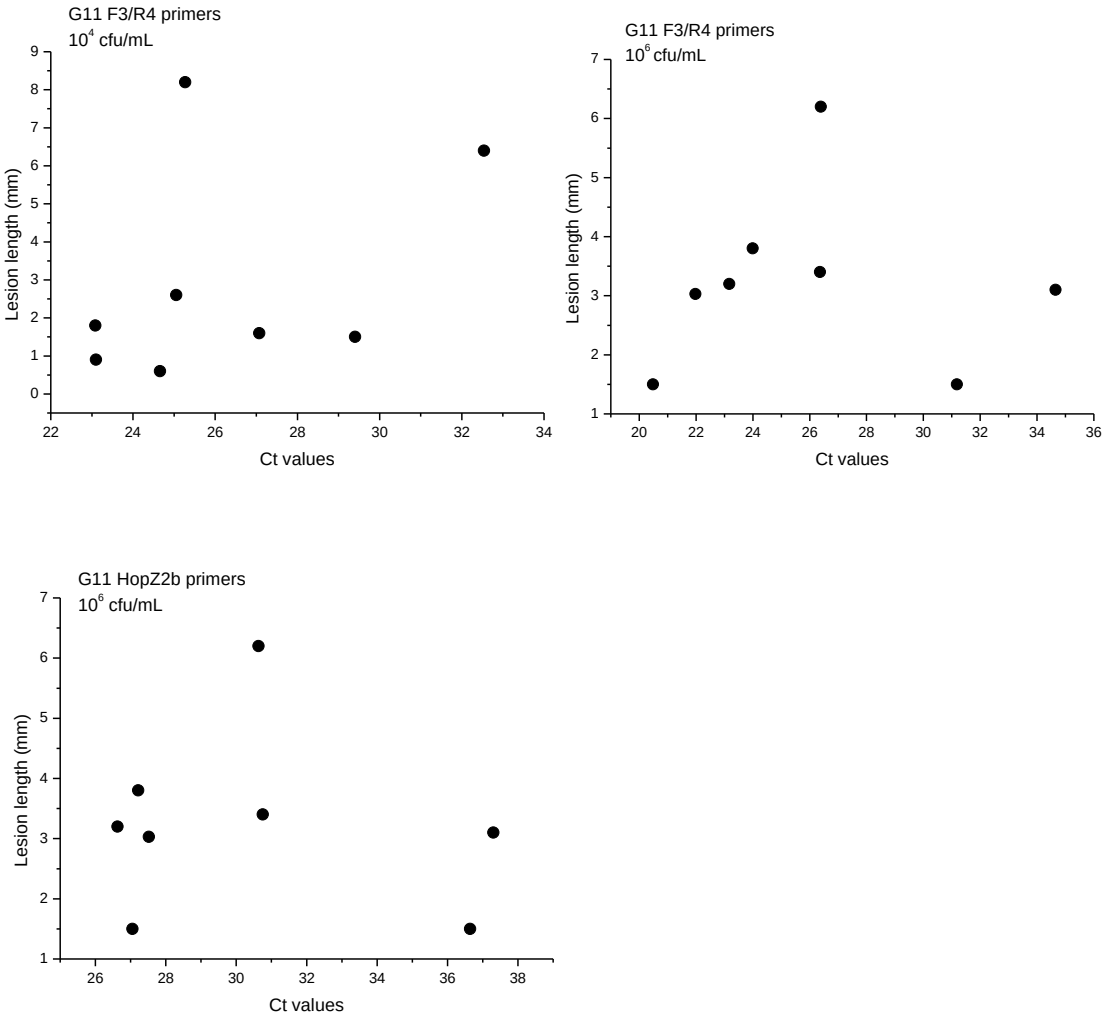








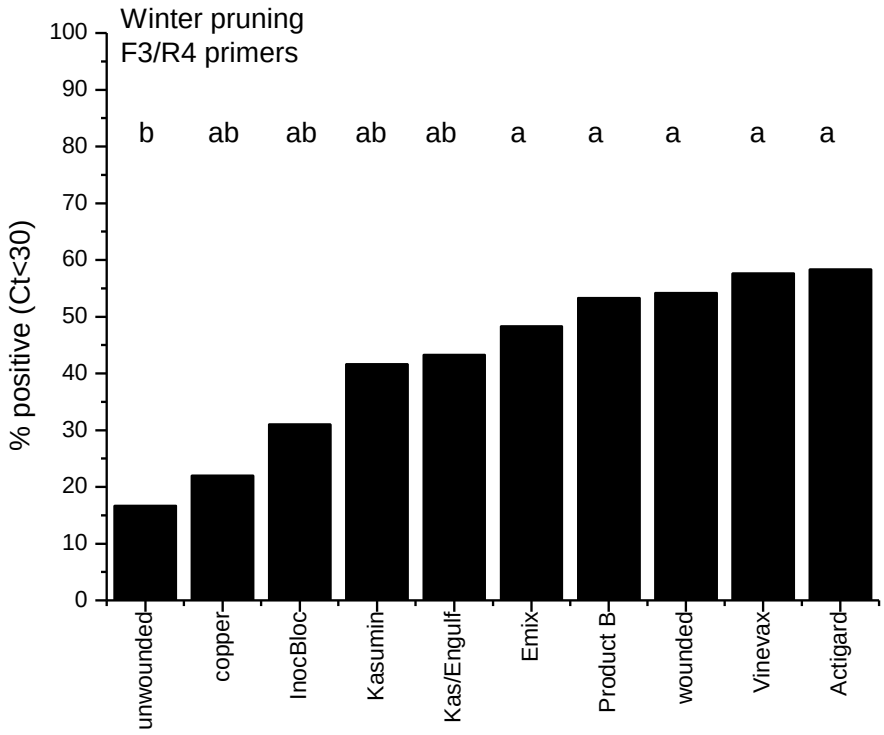




Appendix 3. Lesion lengths plotted against Ct values from qPCR reactions with HopZ2b and F3/R4 primers for *Actinidia chinensis* var. *deliciosa* 'Zes007' G11, *A. chinensis* var. *chinensis* x *A. chinensis* var. *deliciosa* 'Zesh004' G14, *A. chinensis* var. *chinensis* 'Hort16A'kiwifruit canes sampled 11 weeks after wounding, application of wound protectants and inoculation with Psa-V. Potted plants were spray inoculated with either 10^4 or 10^6 cfu/mL Psa-V immediately after wounding and application of wound protectants.

Appendix 4. Baseline Ct values taken from 60 canes (9 treatments x 5 canes per vine) on each of 12 *Actinidia deliciosa* 'Chieftain' kiwifruit vines in the field immediately before application of wound protectants in winter.

Winter pruning	Ct values	
Primers	F3/R4	HopZ2b
	14.56	16.14
	16.12	18.83
	16.69	20.92
	17.8	21.13
	17.88	22.6
	18.46	23.9
	19.24	25.29
	20.27	27.61
	21.02	28.08
	21.83	29.28
	22.82	29.95
	24.98	
	26.81	
	26.85	
	27.06	
	27.23	
	29.2	
	29.31	
	29.59	
No. of positives	19	11
Total no. of samples	540	540
% positives	3.5	2.0



Appendix 5. Untransformed incidence data for numbers of *Actinidia deliciosa* 'Chieftain' kiwifruit canes naturally infected with Psa-V (Ct<30; n = 60) following wounding in winter, application of protectants, and assessment 2 weeks later. Values followed by the same letters are not significantly different according to Tukey's test at α = 0.05 following statistical analysis of arcsine transformed data. Treatments are unwounded = unwounded untreated controls, Kas/Engulf = Kasumin + Engulf®, wounded = wounded untreated controls, copper = copper paste.

Appendix 6. Statistically significant differences between Ct values of 12 vines treated with wound protectants in winter. Means were separated by Tukey's test at α = 0.05.

Primer F3/R4

vine	N	Mean	Grouping
10	50	39.0	A
5	50	34.3	A B
11	50	34.0	A B
7	49	31.9	B C
9	49	31.7	B C
1	49	31.3	B C
12	50	30.9	B C
3	49	30.2	B C D
4	49	28.8	C D
8	50	28.7	C D
2	50	28.0	C D
6	50	25.1	D

Means that do not share a letter are significantly different.

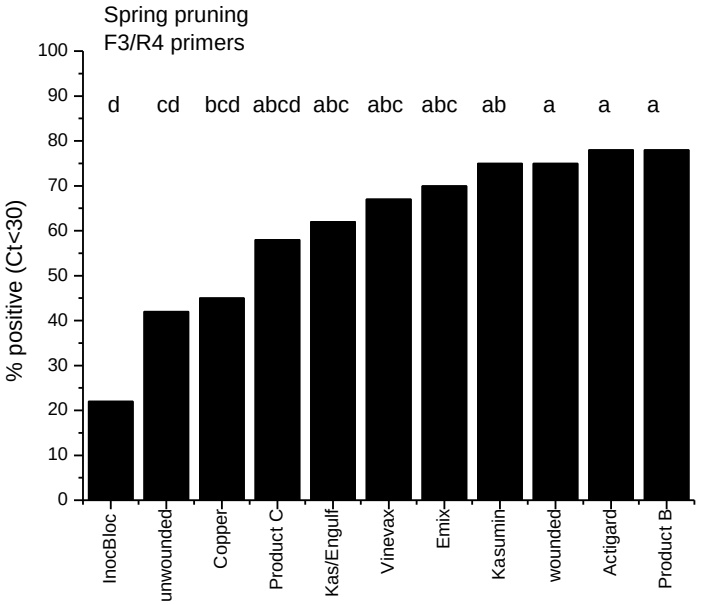
Primer HopZ2b

vine	N	Mean	Grouping
10	50	39.2	A
5	50	38.6	A B
11	50	37.3	A B C
12	50	37.1	A B C
9	49	35.8	A B C D
1	49	35.1	B C D
7	49	34.4	C D
4	49	33.9	C D
3	49	33.8	C D
8	50	33.7	C D
2	50	32.5	D E
6	50	29.8	E

Appendix 7. Baseline Ct values taken from 50 canes (10 treatments x 5 canes per vine) on each of 6 *Actinidia deliciosa* 'Chieftain' kiwifruit vines in the field immediately before application of wound protectants in spring.

Ct values		
Primers	**F3/R4**	**HopZ2b**
	16.75	22.81
	17.94	24.73
	19.10	25.19
	19.96	25.86
	20.01	26.64
	20.69	26.85
	20.75	27.27
	20.81	27.33
	21.50	27.78
	21.64	28.04
	21.69	28.61
	21.75	29.70
	23.63	29.78
	23.74	
	23.90	
	24.74	
	24.90	
	24.92	
	25.30	
	25.32	
	25.54	
	25.64	
	25.79	
	26.67	
	26.85	
	26.93	
	29.17	
	29.46	
No. of positives	28	13
Total no. of samples	300	300
% positives	18	11

Appendix 8. Untransformed incidence data for numbers of *Actinidia deliciosa* 'Chieftain' kiwifruit canes naturally infected with Psa-V (Ct<30; n = 30) following wounding in spring, application of protectants, and assessment 2 weeks later. Values followed by the same letters are not significantly different according to Tukey's test at α = 0.05 following statistical analysis of arcsine transformed data. Treatments are unwounded = unwounded untreated controls, Kas/Engulf = Kasumin + Engulf®, wounded = wounded untreated controls, copper = copper paste.



Appendix 9. Statistically significant differences between Ct values of 6 vines treated with wound protectants in spring. Means were separated by Tukey's test at α = 0.05.

F3/R4 primers

vine	N	Mean	Grouping
1	55	29.9	A
5	55	24.9	B
4	55	23.4	B C
6	55	22.9	B C
2	55	20.8	C
3	55	19.7	C

Means that do not share a letter are significantly different.

HopZ2b primers

vine	N	Mean	Grouping
1	55	33.4	A
5	55	29.3	B
6	55	27.6	B C
4	55	27.6	B C
2	55	25.7	C
3	55	24.7	C

Means that do not share a letter are significantly different

DISCOVER. INNOVATE. GROW.