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Monitoring effectiveness of wound protectants against Psa: Part 2

Everett KR, Shahjahan K, Pushparajah IPS, Ramos L, Parry B, Hasna L, Middleditch C, Vergara MJ

December 2017















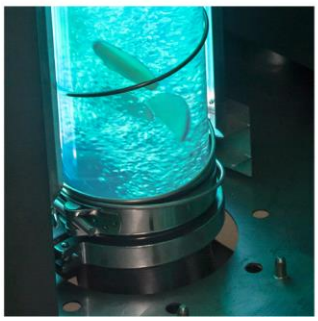


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Report approved by:

Kerry Everett
Scientist/Researcher, Epidemiology and Disease Management
December 2017

Suvi Viljanen
Science Group Leader, Plant Pathology
December 2017

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EXECUTIVE SUMMARY

Monitoring effectiveness of wound protectants against Psa: Part 2

Everett KR[1], Shahjahan K[1], Pushparajah IPS[1], Ramos L[1], Parry B[2], Hasna L[1], Middleditch C[1], Vergara MJ[1]
Plant & Food Research: [1]Auckland, [2]Te Puke

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Introduction

Two new pruning products used on 'Chieftain' rootstocks and non-commercial kiwifruit varieties last season looked promising for protecting wounds from infection by *Pseudomonas syringae* pv. *actinidiae* (Psa). This season seven wound protectants, including these two promising products, were tested on *Actinidia chinensis* var. *deliciosa* 'Hayward' and *A. chinensis* var. *chinensis* 'Zesy002' (commonly known as Gold3) kiwifruit.

Methods

Seven wound protectants (InocBloc™ paste and new formulation, Damar® biological, copper paste, Nordox™ spray, Greenseal™ Ultra and Topsin®) were tested by applying to wounds on potted plants of 'Hayward' and Gold3, and to wounds during winter and spring pruning of 'Hayward' and Gold3 in the orchard. In addition, InocBloc paste and copper paste were applied to girdling wounds in late summer and to grafts in winter, and results were compared with those from the normal grower practice, which was a light spray with Nordox on girdles and grafting wounds were covered with paraffin wax.

Potted plant experiments

- InocBloc paste and new formulation were effective wound protectants on both 'Hayward' and Gold3 potted plants.
- Copper paste was effective on Gold3 pruning wounds.
- Damar biological showed some efficacy on 'Hayward', but not on Gold3.
- Topsin and Greenseal were the least effective wound protectants when compared with uninoculated controls, copper paste, InocBloc paste and new formulation.

Winter pruning

Natural infections were too low in the winter pruning trial for testing the effectiveness of wound protectants.

Spring pruning

- On both 'Hayward' and Gold3 copper paste was the most effective treatment, followed by the two InocBloc formulations.
- In spring a spray with Nordox was not effective as a wound protectant, nor were Topsin, Damar biological or Greenseal.

Girdling wounds

- Current grower practice (light spray with Nordox) was sufficient to prevent infections of the girdling wounds, and to allow fast healing of the wounds.
- Callus formation was not obviously inhibited by the application of Nordox to the girdling wounds (grower practice).
- Girdle wounds were clearly not protected by copper paste.
- Application of InocBloc paste resulted in swelling and cracking of the bark which allowed infection by Psa.

Grafting wounds

- Current grower practice (petroleum jelly) was effective at protecting grafts made during winter, and all five grafts survived and produced fruit the following spring.
- Application of copper paste killed all the grafts it was applied to.
- Application of InocBloc paste killed two of five grafts.

Conclusions

Overall, InocBloc paste and new formulation were the most consistently effective wound protectants when used on pruning wounds, but **InocBloc paste should not be applied to girdling wounds or grafts**. Copper paste appeared to be the most effective wound protectant on pruning cuts, except when inoculum was applied as an aliquot rather than as a spray. **Copper paste should not be applied to girdling wounds or grafts**. Application of this concentrated form of copper killed two out of the five treated vines, and all five grafts.

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 -21-2268133
Fax: +64 9 925 7001
Email: Kerry.Everett@plantandfood.co.nz

1 INTRODUCTION

Wound protection to prevent infection by the virulent strain of *Pseudomonas syringae* pv. *actinidiae* (Psa) continues to be a challenge for the industry. However, a trial completed in March 2016 (Everett et al. 2016) showed that two products consistently resulted in a significant decrease in the amount of Psa in wounds, compared with untreated controls: copper paste (copper sulphate pentahydrate) and InocBloc™ paste. All other products tested, including products already available to growers for wound protection, did not have a significant effect. Genotypes used in that 2016 trial were *Actinidia chinensis* var. *chinensis* 'Hort16A', another green variety and *A. chinensis* var. *chinensis* x *A. chinensis* var. *deliciosa* 'Zesh004' (commonly known as Green14) potted plants and 'Chieftain' males. These promising products needed to be trialled further on the commercially important cultivars *A. chinensis* var. *chinensis* 'Zesy002' (commonly known as Gold3) and *A. chinensis* var. *deliciosa* 'Hayward'. The opportunity was taken to expand the trial and include girdling and grafting wounds, to ensure product safety if used in these situations.

2 METHODS

2.1 Potted Plants

Seven products (Table 1) were tested on 10 replicates of each of Gold3 and 'Hayward' potted plants. There were also untreated controls, and wounded controls, a total of 90 plants per kiwifruit variety. The Gold3 plants were 1-year-old seedlings that arrived at the Te Puke Research Centre (Plant & Food Research; PFR) from Waimea Nursery, Nelson on 25 January 2017. Treatments were applied on 2 February to Gold3 plants, and samples were taken after 16 weeks on 26 May 2017. The 'Hayward' plants were of mixed ages, because grafts made on 1-year-old 'Bruno' rootstocks from Waimea Nursery on 26 January 2017 took on only 29 plants, and were used for three of the ten replicates. To supplement these plants, 27 x 4-month-old ex-tissue culture plants about 0.5 m high were used for three further replicates. Two replicates were 4-year-old plants 2 m high and another two replicates were 3-year-old plants c. 0.5 m high. Treatments were applied to 'Hayward' plants on 4 October 2017, and samples were taken after 6 weeks on 15 November.

Gold3 plants were cut through the main stem at 1 m height with sterilised secateurs. A 5-mm long cane segment was excised from the cut edge and placed in a small plastic ziplock bag for DNA extraction and polymerase chain reaction (PCR) tests to determine a baseline Psa amount. 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. After treatments had dried, wounds were spray-inoculated with 10^6 cfu/mL Psa on 2 February 2017.

Treatments were applied to 'Hayward' plants after cutting the main stem to 1 m high for plants that were over 1 m high, and after cutting about 5 cm below the tops of the shorter plants following the same methodology as for Gold3, including collecting 0.5-cm samples from the cuts for establishing a baseline Psa.

Immediately after application of the pruning products listed below, ten plants of Gold3 for each treatment were spray inoculated with a 10^6 cfu/mL suspension of Psa, and ten plants of 'Hayward' were inoculated with a 10-µL aliquot of 10^6 cfu/mL of Psa. There was a wounded uninoculated control. The reason that 'Hayward' plants were inoculated with a 10-uL aliquot was because permission was granted by the Ministry of Primary Industries to use this method in the field, and therefore the method was simpler and less time-consuming than spray inoculations.

Treatments applied to Gold3 and 'Hayward' were the chemicals described in Table 1:

1. Copper paste (copper sulphate pentahydrate)
2. Copper spray (Nordox™ 75WG)
3. Damar® biological spray
4. Greenseal™ Ultra
5. InocBloc™ new formulation
6. InocBloc paste
7. Topsin® M-4A
8. Inoculated wounded control
9. Non-inoculated wounded control.

The plants were placed in a shaded standing out area in a completely randomised block design and were individually trickle irrigated (Figures 1 and 2). The plants were examined weekly for development of Psa symptoms.

After 16 weeks (26 May 2017), samples of Gold3 plant tissue at the wound site and at 5 and 10 cm below the wound site were taken to be analysed by qPCR for amounts of Psa present. At the same time, the amount of callus formation was recorded. A further set of plants were allowed to heal: callus formation and plant survival was recorded after 3 months.

After 6 weeks (15 November 2017), samples of 'Hayward' plant tissue at the wound site and at 5 and 10 cm below the wound site were taken to be analysed by qPCR for amounts of Psa present. At the same time, the amount of callus formation was recorded. A further set of plants were allowed to heal: callus formation and plant survival was recorded after 3 months on 17 December.

Block 20 seedling layout: TreatmentNumber.RepNumber

Shelter

1.1		3.5	
	9.2		4.10
2.1		5.6	
	6.9		7.6
5.9		9.1	
	7.3		3.2
4.9		2.2	
	3.3		2.3
8.9		1.3	
	5.4		8.6
4.8		6.5	
	4.6		6.6
5.8		7.4	
	1.5		9.6
3.6		8.8	
	8.7		1.10
6.7		4.5	
	2.4		5.1
1.4		9.3	
	9.4		7.10
6.8		7.2	
	4.7		6.2
7.5		3.4	
	3.7		4.2
2.8		6.4	
	5.5		8.3
8.4		2.6	
	4.1		3.1
1.8		5.3	
	6.3		9.7
5.2		4.4	
	7.9		1.9
8.5		8.1	
	3.8		2.7
2.5		1.6	
	9.5		5.10
6.1		3.10	
	5.7		9.9
7.7		7.8	
	1.7		2.10
3.9		9.10	
	2.9		8.10
9.8		7.1	
	8.2		6.10
4.3		1.2	

Shelter

Shelter

Gold3

Hayward

Treatment		
No.	Type	Colour
1	Copper paste	Blue
2	Nordox 75WG	Orange
3	Pruning Sealant Aerosol - Damar	Orange/White
4	Greenseal	Green
5	InocBloc new formulation	Yellow/Black
6	InocBloc Paste	Yellow
7	Topsin	Pink
8	Inoculated wounded control (-ve)	Pink/Black
9	Uninoculated wounded control	White

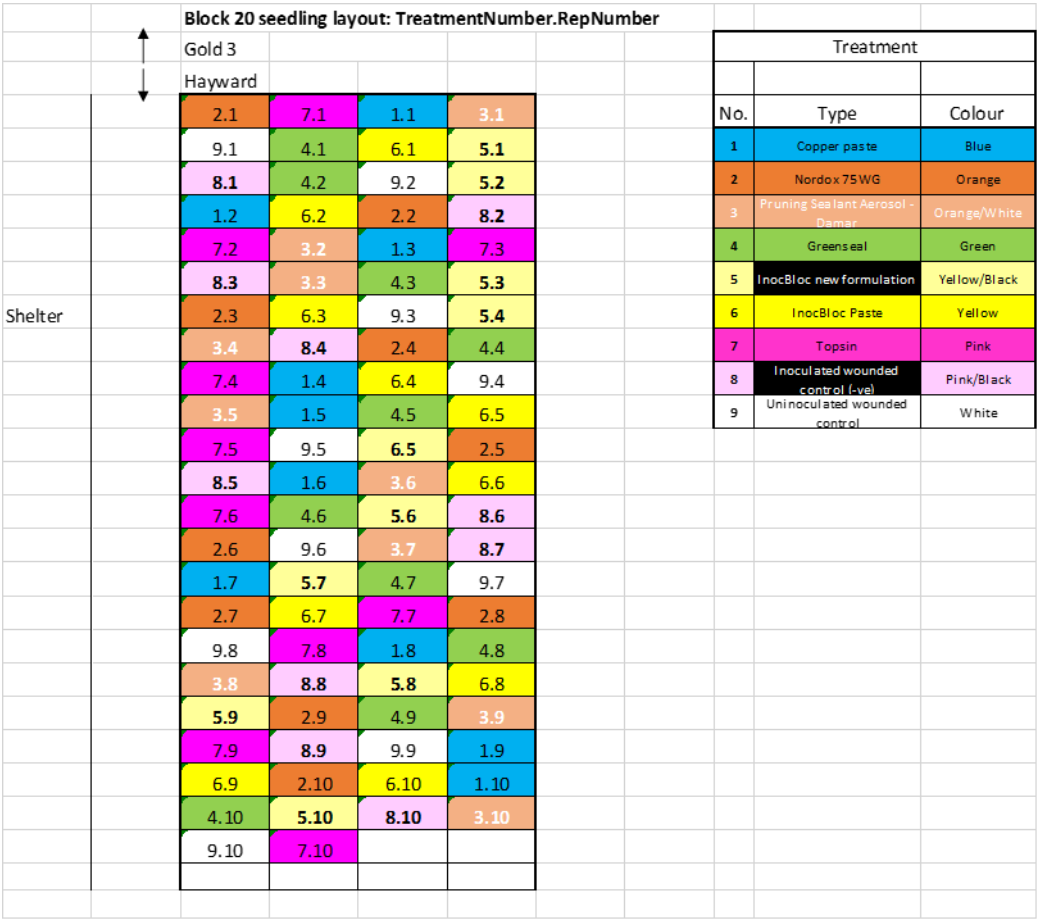

Figure 1. Completely randomised block experimental layout on Block 20 of Te Puke Research Centre of Plant & Food Research for *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) (upper) and *A. chinensis* var. *deliciosa* 'Hayward' (lower) potted plants treated with wound protectants on 2 February and 4 October 2017 respectively.

Figure 2. Treated kiwifruit plants *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) (left) and *A. chinensis* var. *deliciosa* 'Hayward' (right) in a completely random block design under shade on Block 20 in the Te Puke Research Centre orchard of Plant & Food Research.

Table 1. Chemicals used to treat kiwifruit pruning wounds.

Trade name	Active ingredient (a.i.)	% a.i.	Application rate	Application method
Copper sulphate pentahydrate	Copper sulphate pentahydrate	250 g/L (25%)	9.4 kg/L	Paint
Nordox™ 75WG	Cuprous oxide	750 g/kg	7 g/L	Spray
Greenseal™ Ultra	Tebuconazole + octhilinone	10 g/L + 17.5 g/L	undiluted	Paint
Topsin® M-4A	Thiophanate-methyl	400 g/L	1 mL/L	Spray
Damar® biological	Not supplied	Not supplied	Not supplied	Spray
InocBloc™ new formulation	Pine tar	c. 45% pine tar/c. 45% ethanol	undiluted	Paint
InocBloc™ paste	Pine tar	>90%	undiluted	Paint

InocBloc™ is a trademark of Safesan Co. Ltd, Topsin® is a registered trademark of Mitsui & Co. Ltd, Greenseal™ is a trademark of Omnia Specialities NZ Ltd, Nordox™ is a trademark of Nordox AS, Norway. Damar is a registered trademark of Damar Industries Limited, Rotorua.

2.2 Mature Vines

2.2.1 Winter pruning

Eleven-year-old ‘Hayward’ and Gold3 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, except for Nordox 75WG, which was applied as a concentrated paste. ‘Hayward’ and Gold3 vines were treated on 4–5 August 2016. There were six vines, and five canes were treated on each vine with seven wound protectants (Figures 3 and 4), a total of 30 canes per treatment per cultivar. There was a wounded and an unwounded control. In total there were 540 cane samples (270 per cultivar). Treatments were the same as for potted plants (Table 1).

Figure 3. *Actinidia chinensis* var. *deliciosa* ‘Hayward’ (left) and *A. chinensis* var. *chinensis* ‘Zesy002’ (Gold3) (right) kiwifruit vines pruned and treated with wound protectants on 4–5 August 2016, with points where cuts were made indicated by coloured streamers.

On 4 and 5 August a 0.5-cm 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. Two weeks later on 18 August, 0.5-cm cane samples at the wound site and at 5 and 10 cm below the wound site were taken with sterilised secateurs and placed in small ziplock bags for DNA extraction and qPCR analysis. The amount of callus formation was also recorded.

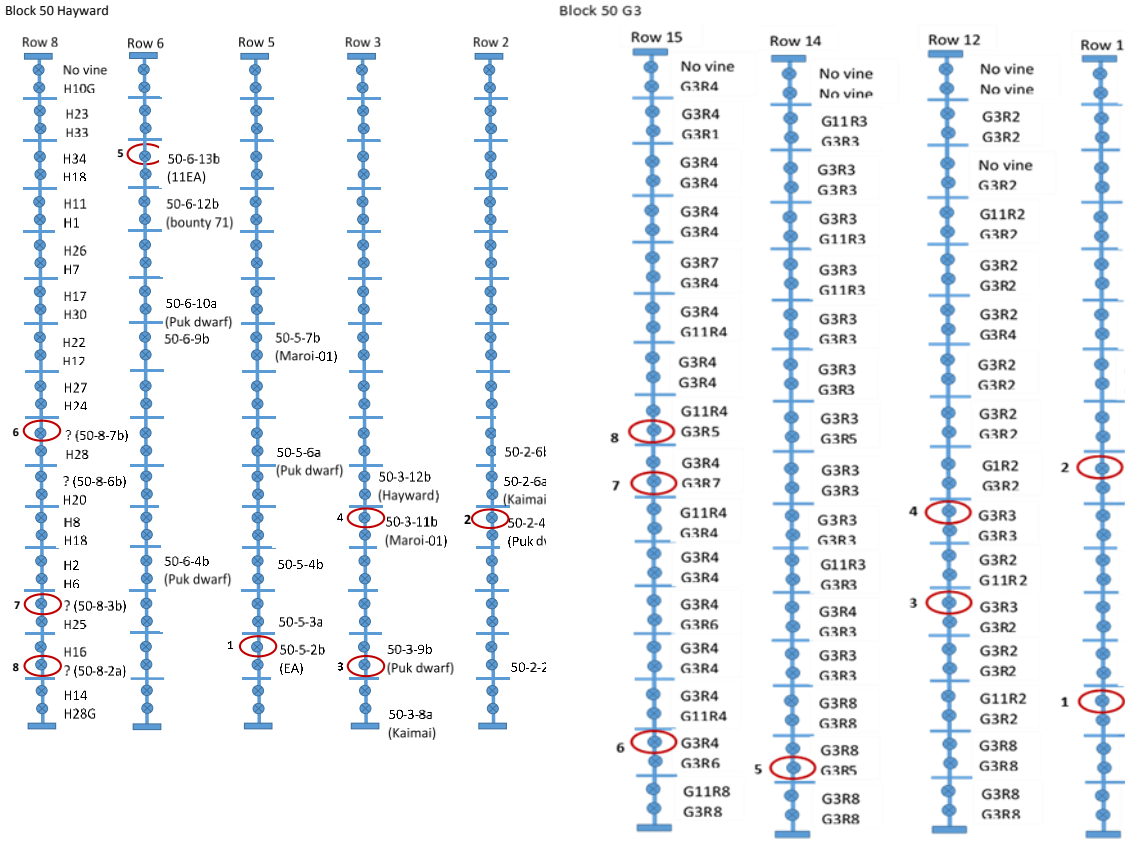

Figure 4. Layout of Block 50 at Te Puke Research Centre orchard and the location of the kiwifruit vines that were sampled (indicated with a red circle). Left diagram is for *Actinidia chinensis* var. *deliciosa* 'Hayward' and right diagram is for *A. chinensis* var. *chinensis* 'Zesy002' (Gold3).

2.2.2 Spring pruning

Eleven-year-old Gold3 kiwifruit vines on Block 50, and 42-year-old 'Hayward' vines on Block 20, 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 Table1. 'Hayward' and Gold3 vines were treated on 8–9 and 15 November 2017. There were six vines, and five canes were treated on each vine with seven wound protectants (Figures 5 and 6), a total of 30 canes per treatment, per cultivar. There was a wounded and an unwounded control. In total there were 540 cane samples, 270 from each cultivar. Treatments were the same as for potted plants (Table 1). Samples for DNA extraction were removed before application of pruning products on November 8–9 and 15, from all treatments except for the unwounded control, and then after 2 weeks on 29 November, as described above.

Figure 5. *Actinidia chinensis* var. *deliciosa* 'Hayward' (left) and *A. chinensis* var. *chinensis* 'Zesy002' (Gold3) (right) kiwifruit vines pruned and treated with wound protectants on 8–9 and 15 November 2017, with points where cuts were made indicated by coloured streamers.

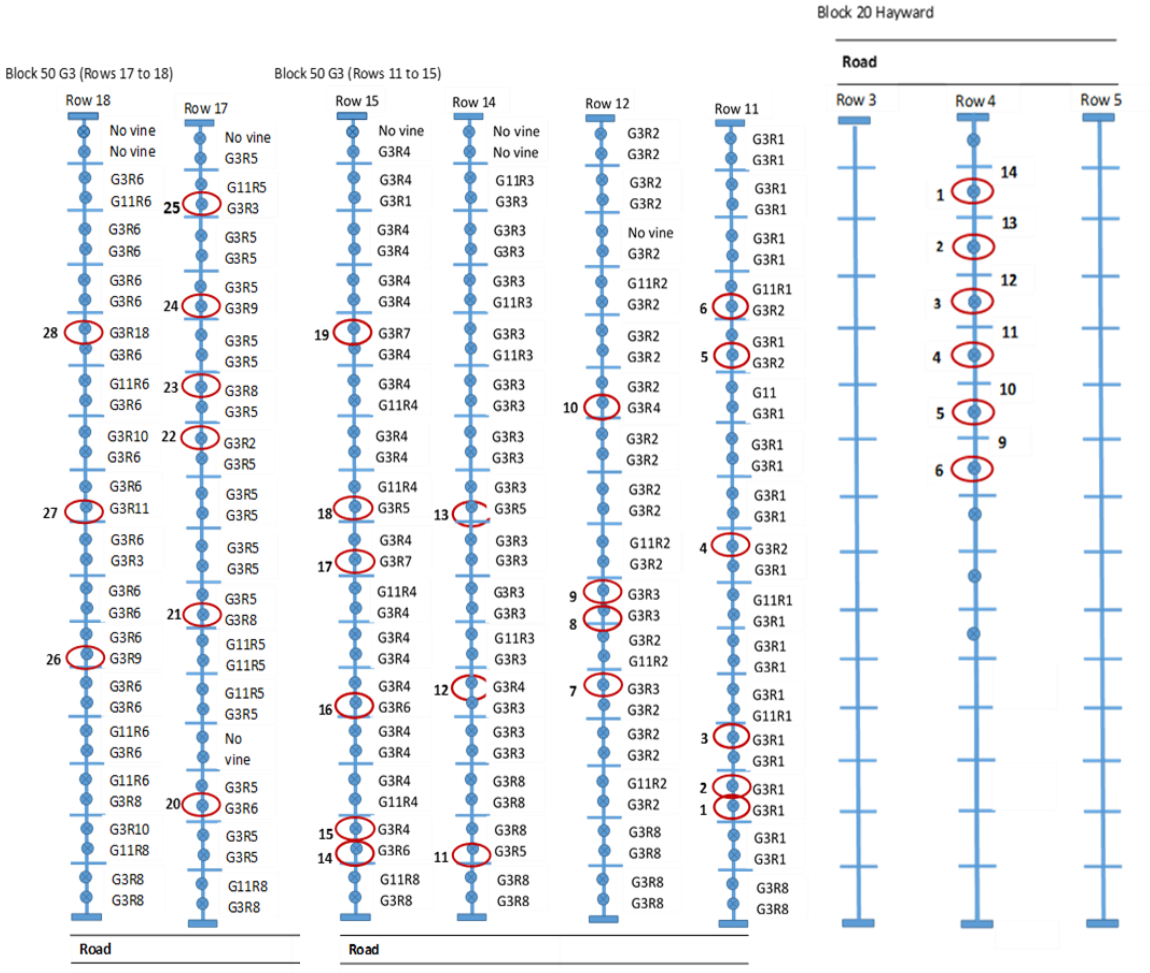

Figure 6. Layout of Block 20 and Block 50 at Te Puke Research Centre orchard and the location of the kiwifruit vines that were sampled (indicated with a red circle). Block 50 (left) was of *A. chinensis* var. *chinensis* 'Zesy002' (Gold3) and block 20 (right) was of *Actinidia chinensis* var. *deliciosa* 'Hayward' kiwifruit vines.

2.3 Girdling wounds

Girdles were applied with a 3/16" girdling knife to 15 Gold3 stump grafted vines on Bruno (2011–12) by a professional horticulturist on a grower orchard in the Bay of Plenty (Figure 7) on 23 February 2017. The vines were distributed in five rows (Figure 8) and treatments were assigned using random numbers.

Figure 7. *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) kiwifruit showing vines, girdling technique, girdles and a girdle treated with copper paste.

The girdling wounds were sampled by drilling with a 1/16" drill bit to a depth of 0.5 cm at four equidistant locations around the trunk. The shavings were collected in bacteriological saline (BS) in an Eppendorf tube (Figure 9). After every use the drill bits were cleaned and washed in 95% ethanol, then air dried on tissue paper. Samples were transferred to the Mt Albert Research Centre laboratories of PFR where a 100-µL aliquot of the bacteriological saline was spread on King's Medium B (King et al. 1954). DNA was extracted from the resultant colonies after 3 days of growth at 28°C according to the methods described in Section 2.5. qPCR was performed as described in Section 2.6 to establish baseline amounts of Psa.

After establishment of girdles and sampling was completed, wound protectants were applied to 10 vines. The protectants applied were InocBloc paste and copper paste (Table 1) according to the randomised complete block design described in Figure 8. There was a grower standard control, which was to spray with Nordox at the rates described in Table 1.

x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x
8	7	6	5	4	3	2	1

Figure 8. *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) kiwifruit vines for which wound protectant was applied to the girdles. X = kiwifruit vine. Row numbers are indicated in the bottom row of the figure. Yellow = grower standard control, green = copper paste and pink = InocBloc™ paste. The green box represents a shade cloth separating the two orchard blocks.

Figure 9. Girdles were sampled for qPCR examination by drilling to a depth of 0.5 cm with a 1/16" drill bit at four equidistant sites around the girdling wound. The shavings were collected in an Eppendorf tube containing 1 mL of bacteriological saline.

Girdling wounds were sampled again after 3 weeks on 15 March 2017. After 16 weeks on 6 July 2017 the girdling wounds were examined and photographed and the callus formation was scored as a percentage. Further samples were taken for qPCR examination. Final samples were taken from remaining plants (the grower removed two dead plants) after 26 weeks (14 September).

2.4 Grafting

On 21 June 2017 horticulturists at Te Puke Research Centre (PFR) re-grafted 3-year-old 'Bruno' rootstocks with Gold3 as a scion (Figure 10). There were five replicate vines per treatment, and the three treatments were conventional grower practice, copper paste and InocBloc. After the results of the girdling trial, the exposure of cut kiwifruit trunks such as the graft sites to InocBloc and copper paste was considered too risky. The grower practice was to apply petroleum jelly to the wounded top of the scion, and to the graft. The treatment was to apply InocBloc and copper paste to the wounded top of the scion and petroleum jelly to the graft. The treatments were applied to five replicates in a completely random block design (Figure 11). Before application of the treatments, two samples were removed from each rootstock and four samples from each scion with the aid of a drill, as described in Section 2.6, to establish baseline Psa. Samples were also taken 2 weeks after grafting on 5 July 2017, and after 13 weeks on 20 September 2017. DNA was extracted from all samples as described in Section 2.5 and were analysed for amount of Psa present by qPCR, as described in Section 2.6. After 26 weeks on 19 December 2017 grafts were visually inspected and the Biologische Bundesanstalt, Budessortenamt and Chemical industry (BBCH) phenological assessment was made (described in Section 2.7).

Figure 10. Grafting of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) scions onto 'Bruno' rootstocks previously grafted with *A. chinensis* var. *deliciosa* 'Zes007' (Green11).

	vine	
replicate	number	treatment
		x
1	1	x
	2	x
	3	x
2	1	x
	2	x
	3	x
3	1	x
	2	x
	3	x
4	1	x
	2	x
	3	x
5	1	x
	2	x
	3	x
		x

Figure 11. Random block design of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) kiwifruit vines to which wound protectant was applied to the grafted scions. X = kiwifruit vine. Yellow = grower standard control, green = copper paste and pink = InocBloc™ paste.

2.5 DNA extractions

A 1000-µL aliquot of BS was added to the small ziplock bags containing cane segments. The plant material was macerated inside the bag using a pestle, then 800 µL 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, 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 14,000 rpm. A 1 µL aliquot of this suspension was used as a template in qPCR reactions.

2.6 Quantitative Polymerase Chain Reaction (qPCR)

2.6.1 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 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. (2013).

2.7 Phenological growth analysis

To determine any adverse effect of the wound protectants on the growth of the kiwifruit plants, a system for scoring phenological growth stages developed for 'Hayward' kiwifruit by Salinero et al. (2009) was used. The system uses the BBCH (**B**iologische **B**udnesanstalt, Budessortenamt and **Ch**emical industry) scale which is a system for uniform coding and description of phenologically similar growth stages of plant species and uses a two digit number to describe each growth stage. Thus dormancy is assigned the 00 code, and all numbers starting with a zero describe bud development (e.g. 01 = beginning of bud swelling, 03 = end of bud swelling). Leaf development stages are described by numbers starting with a one (e.g. 10 = an open cluster containing a few visible leaves, 11 = visible leaves unfolded and start spreading away from the shoot, 12-18 = two to eight or more leaves unfolded, but not yet at full size, 19 = leaves fully developed). Inflorescence emergence is described by numbers starting with a five (e.g. 51 = inflorescence bud swelling, 53 = flower buds growing) and fruit development stages by numbers starting with a seven (e.g. 71 = fruit about 10% of final size, 75 = fruit about 50% of final size, 79 = fruit about 90% of final size).

The Gold3 potted plants were assessed on 20 October 2017, 8 months after the treatments were applied, and the 'Hayward' potted plants were assessed on 19 December 2017, almost 3 months (11 weeks) after the treatments were applied. For 'Hayward' plants the replicates that were not sampled for PCR analysis were assessed separately to the samples that were sampled. Those plants that were sampled for PCR analysis were wounded again due to the nature of the sampling, and the wound protectants were thus removed. However, for 'Gold3', all the plants were sampled for PCR analysis, but only five replicate plants for each treatment were phenologically assessed.

2.8 Statistical analysis

The data were subjected to the general linear model analysis of variance (Minitab ® Release 16) and means were separated with Tukey's test at a significance level of $\alpha = 0.05$. Incidence data were square root transformed before analysis. Graphs were generated using Microcal® Origin. The linear regression function of Microcal Origin was used to describe relationships.

3 RESULTS

3.1 Potted Plants

3.1.1 Gold3

Wound protectants were applied on 2 February and samples were harvested on 26 May 2017, after 16 weeks, Weekly inspections showed that the progression of Psa infection was slow at this time of year when mean daily temperatures averaged 16.8°C (Appendix 1). When temperatures started to fall, symptoms became more obvious on wounded controls, and a decision was made to harvest the trial in late May.

Table 2. Mean qPCR Crossing threshold (Ct) values and incidence of *Pseudomonas syringae* pv. *actinidiae* (Psa) for *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) potted plants treated with wound protectants and sampled after 16 weeks in a standing out area in the orchard.

	Ct value				Incidence*			
Treatments	F3/R4		HopZ2b		F3/R4		HopZ2b	
1. Copper sulphate pentahydrate	39.2±0.4	a	40	a	20	b	10	b
2. Nordox™ 75WG	39.1±0.4	a	40	a	20	b	0	b
3. Greenseal™ Ultra	35.3±0.7	b	39.2±0.3	a	90	a	40	ab
4. Topsin® M-4A	30.5±1.3	c	35±0.9	b	90	a	80	a
5. Damar® biological	35.3±0.9	b	38.7±0.4	a	70	a	40	ab
6. InocBloc™ new formulation	39.1±0.4	a	39.9±0.1	a	20	b	0	b
7. InocBloc paste	39.5±0.3	a	39.9±0.1	a	10	b	10	b
8. uninoculated control	39.4±0.3	a	40	a	20	b	0	b
9. inoculated control	31.1±1.2	c	36.3±0.9	b	100	a	70	a
ANOVA					*P* value			
Treatment	<0.0001		<0.0001		<0.0001		<0.0001	
Vine	0.314		0.026		0.676		0.815	
Treatment*Vine	<0.0001		<0.0001		0.960		0.267	
Height	<0.0001		<0.0001		0.022		0.003	

*Incidence data were square root transformed before analysis. Numbers in a column with the same letter are not significantly different according to Tukey's test (α <0.05).

Before the treatments were applied, the baseline analysis showed that Psa was detected in fewer than three of the 10 treated plants for most treatments by the F3/R4 primers (Figure 12), but was detected in seven of the 10 vines treated with Greenseal. All treated plants except those treated with Greenseal (four) and copper paste (one) were negative for Psa when tested with the HopZ2b primers.

Analysis of variance of plants sampled after 16 weeks showed that treatment effects were statistically significant, and that there was a significant interaction between treatments and vines (Table 2). There was also a significant effect due to the samples being taken at differing

distances from the wound (0, 5 and 10 cm) (height effect). For all treated plants except for those treated with Greenseal, the amount of Psa detected declined the further from the wound samples were taken (Figure 12).

Significantly less Psa than in the wounded controls was detected for all treatments except Topsin when Ct values were analysed (Table 2). Incidence: Psa was detected in statistically significantly fewer plants than wounded controls when wounds were treated with copper paste, Nordox, InocBloc new formulation and paste (Table 2).

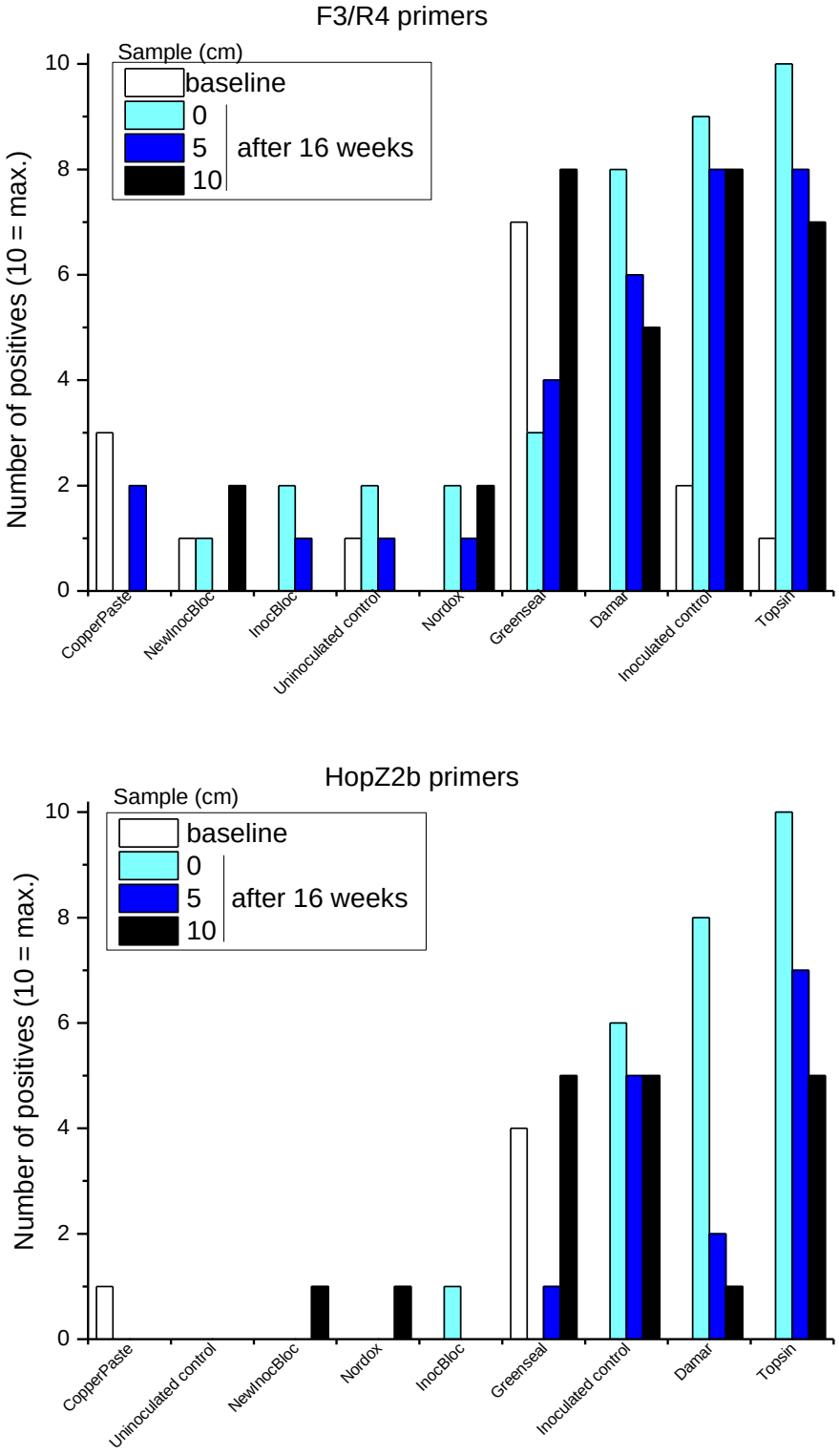

Figure 12. Number of *Pseudomonas syringae* pv. *actinidiae* (Psa)-positive samples detected by qPCR using F3/R4 (above) or HopZ2b (below) primers for *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) potted plants treated with wound protectants. Samples after 16 weeks were taken 0, 5 and 10 cm from the wound.

3.1.2 'Hayward'

Before the treatments were applied, the baseline analysis showed that Psa was detected in only one of the 90 'Hayward' plants used in this experiment (Figure 13).

The progression of the symptoms in wounded 'Hayward' controls was more rapid than that observed in the Gold3 plants. When 'Hayward' potted plants were treated on 4 October the mean daily average temperature was about 3°C lower than when Gold3 potted plants were treated in February (mean daily temperature was 14.1°C, Appendix 2). Therefore, a decision was made to sample from the 'Hayward' plants 6 weeks after treatment.

Analysis of variance of samples after 6 weeks showed that treatment effects were statistically significant, and that there was a significant interaction between treatments and vines (Table 3). There was also a significant vine effect, but this was confounded by vines being different ages. Less Psa was detected in vines that were older (results not shown). There was no significant effect due to the samples being taken at differing distances from the wound (0, 5 and 10 cm) (height effect) (Figure 13).

Those treatments for which significantly less Psa than in the wounded controls was detected with the F3/R4 primers were Topsin, Damar biological, InocBloc new formulation and paste and the uninoculated control (Table 3). Damar biological, InocBloc new formulation and paste were the treatments for which significantly less Psa than in the wounded controls was detected with the HopZ2b primers (Table 3). Incidence: Psa was detected in significantly fewer plants than in wounded controls when wounds were treated with InocBloc new formulation and paste (Table 3).

Table 3. Mean qPCR Crossing threshold (Ct) values and incidence of *Pseudomonas syringae* pv. *actinidiae* (Psa) for *Actinidia chinensis* var. *deliciosa* 'Hayward' potted plants treated with wound protectants and sampled after 16 weeks in a standing out area in the orchard.

	Ct value				Incidence*			
Treatments	F3/R4		HopZ2b		F3/R4		HopZ2b	
1. Copper sulphate pentahydrate	34.3±2.1	cd	37.4±1.4	ab	40	ab	33.3	ab
2. Nordox™ 75WG	35.8±1.9	bcd	37.5±1.3	ab	40	ab	20	bc
3. Greenseal™ Ultra	29.2±2.3	e	33.7±1.8	c	66.7	a	53.3	a
4. Topsin® M-4A	37.0±1.4	abc	38.8±0.8	ab	26.7	bc	13.3	bc
5. Damar® biological	39.7±0.2	a	39.9±0.1	a	13.3	bc	6.7	bc
6. InocBloc™ new formulation	39.8±0.2	a	40	a	6.7	c	0	c
7. InocBloc paste	40	a	40	a	0	c	0	c
8. uninoculated control	38.1±0.8	ab	39.9±0.2	ab	40	ab	6.7	bc
9. inoculated control	33.1±2.3	d	36.5±1.5	bc	40	ab	33.3	ab
ANOVA					*P* value			
Treatment	<0.0001		<0.0001		<0.0001		<0.0001	
Vine	<0.0001		<0.0001		<0.0001		<0.0001	
Treatment*Vine	<0.0001		<0.0001		<0.0001		<0.0001	
Height	0.199		0.875		0.502		0.421	

*Incidence data were square root transformed before analysis. Numbers in a column with the same letter are not significantly different according to Tukey's test (α <0.05).

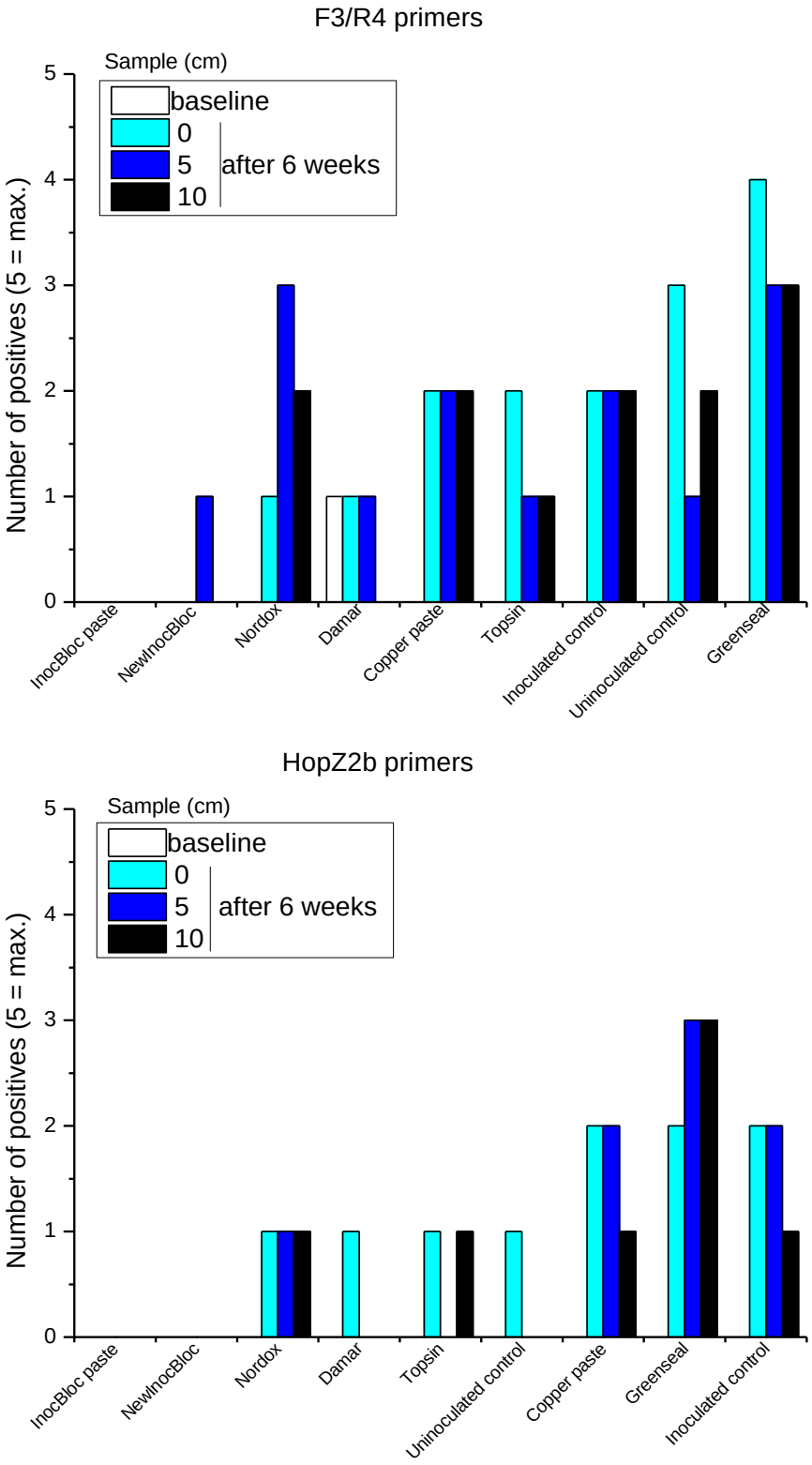

Figure 13. Number of *Pseudomonas syringae* pv. *actinidiae* (Psa)-positive samples detected by qPCR using F3/R4 (above) or HopZ2b (below) primers for *Actinidia chinensis* var. *deliciosa* 'Hayward' potted plants treated with wound protectants. Samples after 6 weeks were taken 0, 5 and 10 cm from the wound.

3.2 Mature Vines

3.2.1 Winter pruning

'Hayward'

The baseline analysis of samples taken when the wound protectants were applied on 4–5 August 2017 showed that incidence of Psa was fewer than six of 30 samples taken per treatment when F3/R4 primers were used, and 10 or fewer of 30 samples when HopZ2b primers were used (Figure 14).

There was no significant treatment effect on incidence of Psa detected by the F3/R4 primers when samples were taken after 2 weeks on 18 August (Table 4), and there was no significant effect of sampling time. However, incidence was low, Psa being detected in six or fewer of 30 samples per treatment by the F3/R4 primers (Figure 14), even 2 weeks after treatment.

There was a significant treatment effect on incidence when the HopZ2b primers were used (Table 4), but no effect of sampling time. When the differences between treatments for samples taken 2 weeks after treatment were analysed, there were no significant differences between treatments when either PCR primer set were used (Table 5).

The treatment effect for Ct values was significant when both primer sets were used (F3/R4 and HopZ2b), and there was a significant difference for time of sampling (Table 4). However, none of the treatments resulted in a significantly lower detection of Psa than in wounded controls with either primer set (Table 5).

Temperatures during this period in August were low, the mean daily temperature during this 2-week period being 11.8°C (Appendix 3).

Table 4. Mean qPCR Crossing threshold (Ct) values and incidence of *Pseudomonas syringae* pv. *actinidiae* (Psa) for *Actinidia chinensis* var. *deliciosa* 'Hayward' vines treated with wound protectants and sampled after 2 weeks.

	Ct value		Incidence*	
	F3/R4	HopZ2b	F3/R4	HopZ2b
Treatment	0.001	<0.0001	0.477	0.004
Vine	<0.0001	<0.0001	<0.0001	<0.0001
Treatment*Vine	<0.0001	<0.0001	0.659	0.104
Sampling time	<0.0001	0.036	0.159	0.869

*Incidence data were square root transformed before analysis.

Table 5. Mean qPCR Crossing threshold (Ct) values and incidence of *Pseudomonas syringae* pv. *actinidiae* (Psa) for *Actinidia chinensis* var. *deliciosa* 'Hayward' vines treated with wound protectants and sampled after 2 weeks.

	Ct value				Incidence*	
Treatments	F3/R4		HopZ2b		F3/R4	HopZ2b
1. Copper sulphate pentahydrate	38.9±0.6	ab	39.6±0.4	ab	17.7	1.7
2. Nordox™ 75WG	40	a	40	a	0	1.7
3. Greenseal™ Ultra	36.3±1.5	ab	38±1.1	abc	23.3	5
4. Topsin® M-4A	35.3±1.7	b	37.2±1.3	bc	23.3	15
5. Damar® biological	36.7±1.5	ab	36.3±1.3	c	17.7	28.3
6. InocBloc™ new formulation	39.3±0.7	a	40	a	3.3	3.3
7. InocBloc paste	37.7±1.3	ab	38.4±0.9	abc	10	6.7
8. unwounded control	38.7±0.9	ab	39±0.7	ab	10	10
9. wounded control	37.8±1.0	ab	38.4±0.8	abc	17.7	18.3
ANOVA				*P* value		
Treatment	0.002		<0.0001		0.204	0.185
Vine	<0.0001		<0.0001		<0.0001	<0.0001
Treatment*Vine	<0.0001		<0.0001			

*Incidence data were square root transformed before analysis. Numbers in a column with the same letter are not significantly different according to Tukey's test (α <0.05).

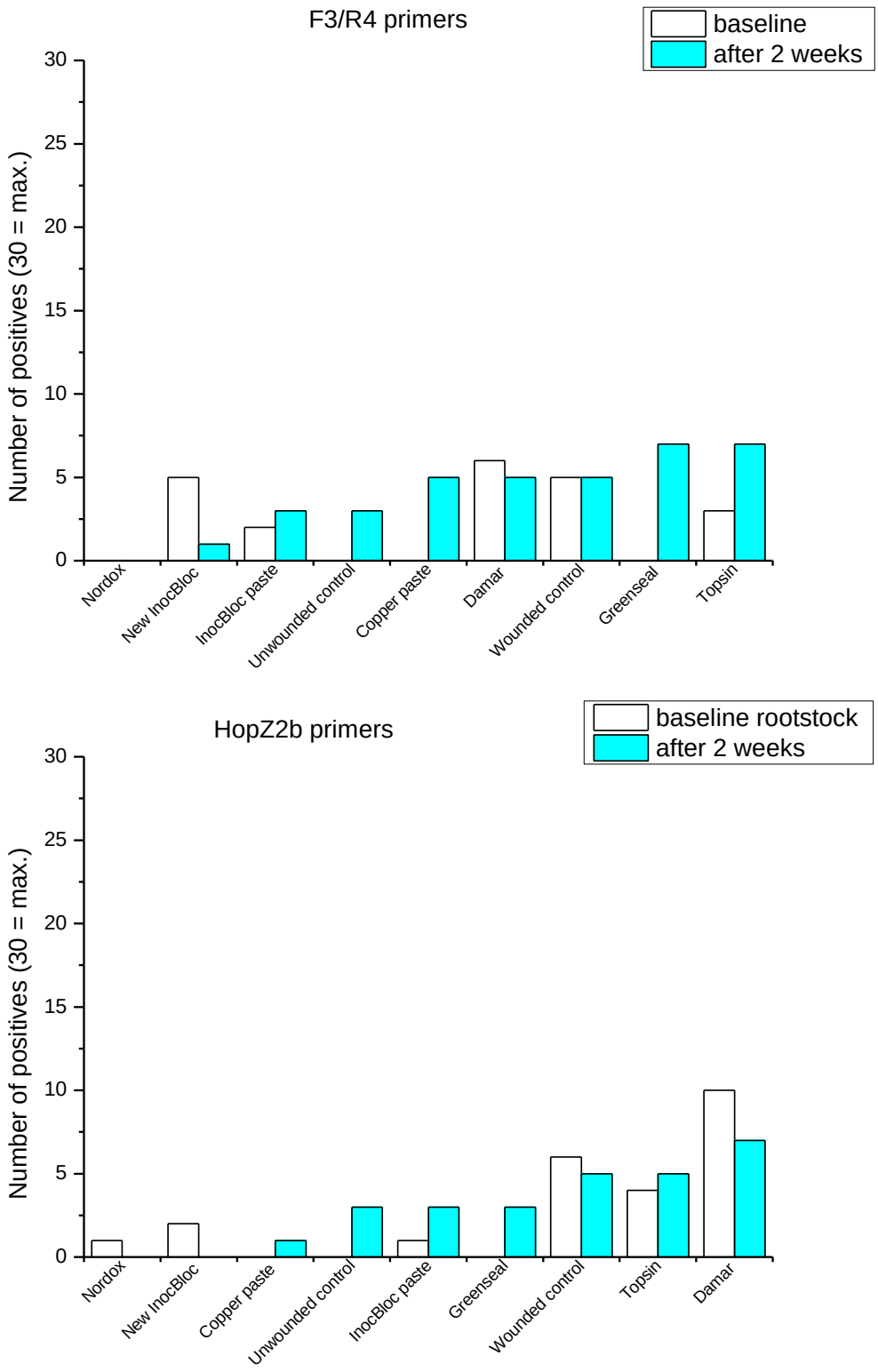

Figure 14. Number of *Pseudomonas syringae* pv. *actinidiae* (Psa)-positive samples by qPCR with F3/R4 (above) and HopZ2b (below) primers for samples taken at the same time as pruning (baseline) and samples taken 2 weeks later after application of protectants in winter to pruning wounds of *Actinidia chinensis* var. *deliciosa* 'Hayward' kiwifruit vines.

Gold3

The analysis of samples taken when the wound protectants were applied on 4–5 August 2017 showed that Psa was detected in four out of the 270 samples when F3/R4 primers were used, and two when HopZ2b primers were used (Figure 15).

There was no significant increase in Psa incidence when samples were taken after 2 weeks on 18 August (Table 6), nor was there a significant decrease in Ct values during this time (Table 6).

The analysis of Ct values for samples taken 2 weeks after treatment showed no significant treatment effect (Table 7). However, incidence remained low, with Psa being detected in eight of the 270 samples when the F3/R4 primers were used, and in three of the 270 samples when the HopZ2b primers were used (Figure 15).

The mean daily temperature during this time was 11.8°C (Appendix 3).

Table 6. Mean qPCR Crossing threshold (Ct) values and incidence of *Pseudomonas syringae* pv. *actinidiae* (Psa) for *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) vines treated with wound protectants and sampled after 2 weeks.

	Ct value		Incidence*	
	F3/R4	HopZ2b	F3/R4	HopZ2b
Treatment	<0.0001	0.001	0.018	<0.0001
Vine	0.156	0.245	0.590	0.006
Treatment*Vine	0.179	0.08	0.544	0.001
Sampling time	0.996	0.552	0.540	1.0

*Incidence data were square root transformed before analysis.

Table 7. Mean qPCR Crossing threshold (Ct) values and incidence of *Pseudomonas syringae* pv. *actinidiae* (Psa) for *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) vines treated with wound protectants and sampled after 2 weeks.

	Ct value		Incidence*	
Treatments	F3/R4	HopZ2b	F3/R4	HopZ2b
1. Copper sulphate pentahydrate	40	40	0	0
2. Nordox™ 75WG	40	40	0	0
3. Greenseal™ Ultra	39.9±0.2	40	3.3	0
4. Topsin® M-4A	38.4±0.8	39.3±0.6	10	6.7
5. Damar® biological	39.9±0.1	40	3.3	0
6. InocBloc™ new formulation	40	40	0	0
7. InocBloc paste	40	40	0	0
8. unwounded control	39.5±0.5	39.9±0.1	3.3	3.3
9. wounded control	39.7±0.4	40	6.7	0
ANOVA		*P* value		
Treatment	0.235	0.233	0.403	0.137
Vine	0.579	0.520	0.452	0.651
Treatment*Vine	0.575	0.590		

*Incidence data were square root transformed before analysis. Numbers in a column with the same letter are not significantly different according to Tukey's test (α <0.05).

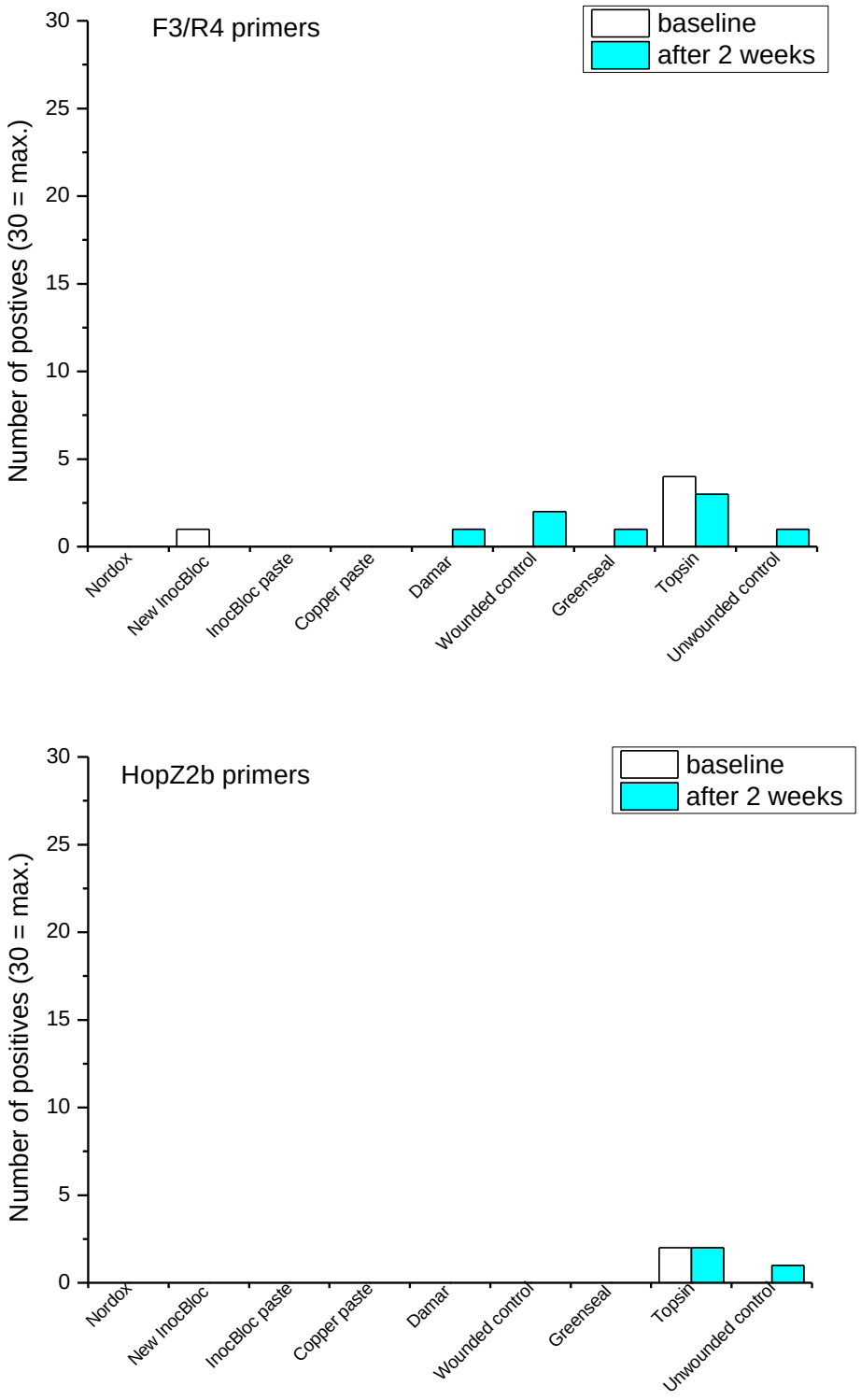

Figure 15. Number of *Pseudomonas syringae* pv. *actinidiae* (Psa)-positive samples by qPCR with F3/R4 and HopZ2b primers for samples taken at the same time as pruning (baseline) and samples taken 2 weeks later after application of protectants in winter to pruning wounds of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) kiwifruit vines.

3.2.2 Spring pruning

The mean daily temperature during the 3 weeks in which this experiment was conducted was 15.7°C (Appendix 5).

'Hayward'

The analysis of samples taken when the wound protectants were applied on 8–9 and 15 November 2017 showed that baseline Psa incidence was 10 of 30 samples or fewer per treatment when F3/R4 primers were used, and six or fewer when HopZ2b primers were used (Figure 16). The incidence of Psa was similar between the treatments.

There was a significant increase in Psa incidence when samples were taken after 3 weeks on 29 November (Table 8). Thus the Ct values were significantly lower when samples were taken 3 weeks after treatment (Table 8). There was a significant treatment effect for both Ct values and incidence (Table 8).

The analysis of Ct values and Psa incidence for samples taken 3 weeks after treatment showed a significant treatment effect (Table 9). There was also a significant vine effect when the HopZ2b primers were used, but there was no significant interaction between treatment and vines (Table 9). Those treatments that resulted in a significantly higher Ct value (less Psa detected) than for wounded controls when both primer sets were used were copper paste and InocBloc new formulation and paste (Table 9). When Psa incidence was analysed, InocBloc paste and copper paste resulted in significantly fewer detections of Psa by the F3/R4 primers, and InocBloc new formulation and copper paste when the HopZ2b primers were used (Table 9, Figure 16).

Table 8. Mean qPCR Crossing threshold (Ct) values and incidence of *Pseudomonas syringae* pv. *actinidiae* (Psa) for *Actinidia chinensis* var. *deliciosa* 'Hayward' vines treated with wound protectants and sampled after 2 weeks.

	Ct value		Incidence*	
Treatments	F3/R4	HopZ2b	F3/R4	HopZ2b
Treatment	<0.0001	<0.0001	0.001	0.037
Vine	0.209	0.009	0.741	0.163
Treatment*Vine	0.191	0.396	0.496	0.654
Sampling time	<0.0001	<0.0001	<0.0001	<0.0001

*Incidence data were square root transformed before analysis

Table 9. Mean qPCR Crossing threshold (Ct) values and incidence of *Pseudomonas syringae* pv. *actinidiae* (Psa) for *Actinidia chinensis* var. *deliciosa* 'Hayward' vines treated with wound protectants and sampled after 2 weeks.

	Ct value				Incidence*			
Treatments	F3/R4		HopZ2b		F3/R4		HopZ2b	
1. Copper sulphate pentahydrate	38.6±0.8	a	39.7±0.3	a	16.7	c	3.3	d
2. Nordox™ 75WG	30.2±1.6	c	33.8±1.1	c	63.3	ab	53.3	a
3. Greenseal™ Ultra	31.2±1.5	bc	35.1±1.0	bc	60	ab	50	a
4. Topsin® M-4A	31.3±1.3	bc	34.7±0.4	bc	73.3	a	56.7	a
5. Damar® biological	31.1±1.2	bc	36.6±0.8	abc	76.7	a	46.7	ab
6. InocBloc™ new formulation	36.0±1.0	ab	39.1±0.5	a	36.7	abc	13.3	bcd
7. InocBloc paste	36.6±1.1	a	38.1±0.7	ab	30	bc	26.7	abc
8. unwounded control	38.1±0.7	a	39.4±0.4	a	23.3	bc	10	cd
9. wounded control	29.4±1.3	c	34.1±1.0	c	80	a	60	a
ANOVA					*P* value			
Treatment	<0.0001		<0.0001		<0.0001		<0.0001	
Vine	0.164		0.001		0.357		0.006	
Treatment*Vine	0.205		0.410					

*Incidence data were square root transformed before analysis. Numbers in a column with the same letter are not significantly different according to Tukey's test (α <0.05).

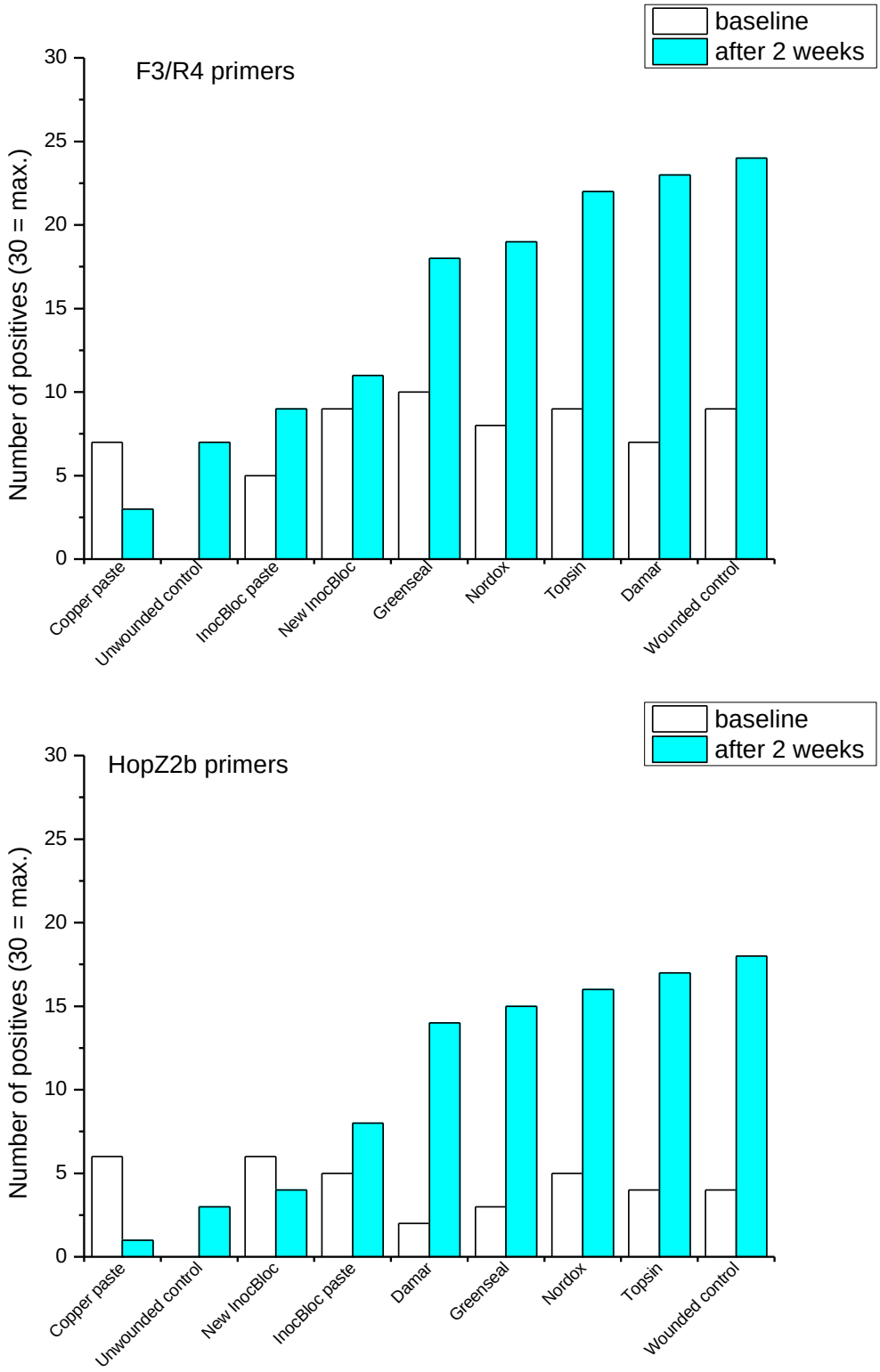

Figure 16. Number of *Pseudomonas syringae* pv. *actinidiae* (Psa)-positive samples by qPCR with F3/R4 (above) and HopZ2b (below) primers for samples taken at the same time as pruning (baseline) and samples taken 2 weeks later after application of protectants in spring to pruning wounds of *Actinidia chinensis* var. *deliciosa* 'Hayward' kiwifruit vines.

Gold3

The baseline analysis of samples taken when the wound protectants were applied on 8–9 and 15 November 2017 showed that the incidence of Psa ranged between 16 and 25 of 30 samples per treatment when F3/R4 primers were used, and between 8 and 14 of 30 samples when HopZ2b primers were used (Figure 17). This incidence was similar between the treatments.

There was a significant increase in Psa incidence when samples were taken after 3 weeks on 29 November; however, the Ct values were not significantly lower (Table 10). There was a significant treatment effect for both Ct values and Psa incidence (Table 10), except for incidence measured with the HopZ2b primers.

The analysis of Ct values and incidence for samples taken 3 weeks after treatment showed a significant treatment effect (Table 11). There was a significant vine effect for Ct values when the HopZ2b primers were used, and there was a significant interaction between treatment and vines (Table 11).

No treatments resulted in a significantly higher Ct value (less Psa detected) than that for wounded controls when both primer sets were used (Table 11). When incidence was analysed, InocBloc new formulation and copper paste resulted in significantly fewer detections of Psa by the F3/R4 primers, but no treatments resulted in fewer detections than wounded controls when the HopZ2b primers were used (Table 11, Figure 17).

Table 10. Mean qPCR Crossing threshold (Ct) values and incidence of *Pseudomonas syringae* pv. *actinidiae* (Psa) for *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) vines treated with wound protectants and sampled after 2 weeks.

	Ct value		Incidence*	
Treatments	F3/R4	HopZ2b	F3/R4	HopZ2b
Treatment	0.001	0.031	0.008	0.252
Vine	0.474	0.009	0.158	0.241
Treatment*Vine	<0.0001	0.001	0.834	0.305
Sampling time	0.052	0.189	<0.0001	0.046

*Incidence data were square root transformed before analysis.

Table 11. Mean qPCR Crossing threshold (Ct) values and incidence of *Pseudomonas syringae* pv. *actinidiae* (Psa) for *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) vines treated with wound protectants and sampled after 2 weeks.

	Ct value				Incidence*			
Treatments	F3/R4		HopZ2b		F3/R4		HopZ2b	
1. Copper sulphate pentahydrate	39.2±0.6	a	39.2±0.5	a	6.7	d	6.7	b
2. Nordox™ 75WG	28.5±1.5	c	32.8±1.4	c	76	a	57	a
3. Greenseal™ Ultra	34±1.5	abc	36.1±1.1	abc	37	abcd	33	ab
4. Topsin® M-4A	32.7±1.8	bc	33.6±1.5	bc	43	abc	40	ab
5. Damar® biological	32±1.8	bc	35.3±1.3	abc	47	ab	37	ab
6. InocBloc™ new formulation	38.7±0.9	a	39.2±0.5	a	6.7	cd	6.7	b
7. InocBloc paste	39.2±0.6	ab	38.5±0.8	a	20	bcd	13	ab
8. unwounded control	36.5±1.1	ab	38±0.8	ab	37	abc	20	ab
9. wounded control	33.4±1.5	abc	36.5±1.0	abc	50	ab	37	ab
ANOVA				*P* value				
Treatment	<0.0001		<0.0001		<0.0001		0.003	
Vine	0.243		<0.0001		0.169		0.113	
Treatment*Vine	0.297		0.029					

*Incidence data were square root transformed before analysis. Numbers in a column with the same letter are not significantly different according to Tukey's test (α <0.05).

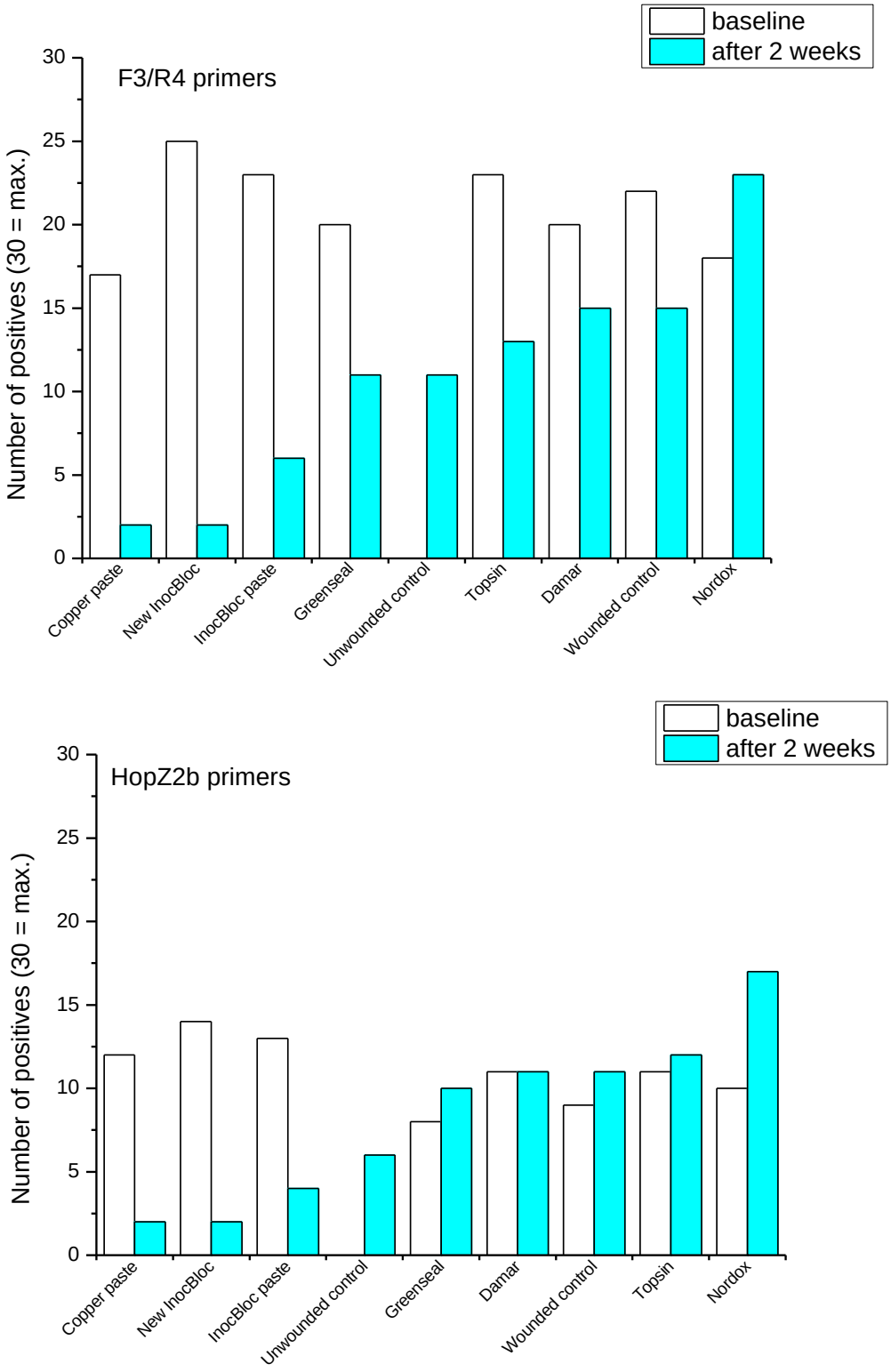

Figure 17. Number of *Pseudomonas syringae* pv. *actinidiae* (Psa)-positive samples by qPCR with F3/R4 and HopZ2b primers for samples taken at the same time as pruning (baseline) and samples taken 2 weeks later after application of protectants in spring to pruning wounds of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) kiwifruit vines.

3.3 Girdling wounds

The analysis by qPCR of the samples from the girdling wounds did not show any consistent differences between treatments until week 26 (Table 12). At this sampling time, 75% of the wounds treated with copper paste, 20% of the wounds treated with InocBloc and none of the wounds treated according to grower practice were positive for Psa when the F3/R4 primers were used. The differences were not statistically significant when Psa incidence was analysed (P = 0.072) or for Ct values (P = 0.138). No Psa was detected with the HopZ2b primers at this time.

Examination of the wounds after 3 weeks showed that wounds treated with InocBloc were swollen (Figure 18a). Wounds treated with copper showed some exudate (Figure 18b) whilst grower-practice wounds showed no unusual symptoms (Figure 18c). Examination of the wounds after 3 months showed that wounds treated with InocBloc produced the most callus (98%, Table 13), and wounds treated with copper paste the least (54%). Two of the vines treated with copper were killed (Figure 19). The wounds treated with InocBloc were swollen, with some cracking of the bark (Figure 20). In contrast, the wounds treated with normal grower practice healed flat (Figure 21), and produced 78% callus (Table 13).

The average mean daily temperature during this trial was 13.4°C (Appendix 5).

Table 12. Percentage *Pseudomonas syringae* pv. *actinidiae* (Psa) positives (max. 5 for weeks 0, 3 and 16, and max. 4 for week 26) for girdling wounds of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) vines treated with copper paste and InocBloc™ tested using two primers F3/R4 and HopZ2b.

	F3/R4			HopZ2b		
	Treatments					
Time (weeks)	copper	grower practice	InocBloc	copper	grower practice	InocBloc
0	0	20	0	0	20	0
3	20	0	20	20	0	20
16	0	0	0	0	0	0
26	75	0	25	0	0	0

Table 13. The effect of different treatments on callusing of girdling wounds on *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3), 16 weeks after establishment.

	Treatment		
Replicate	InocBloc	Copper paste	Grower standard
1	100	100	50
2	100	20	70
3	100	100	80
4	100	5	90
5	90	0	100
Average	98	54	78

a.

b.

c.

Figure 18. Girdling wounds on *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) 3 weeks after treatment a. InocBloc™, b. copper paste c. grower practice.

Figure 19. Copper-treated girdling wounds on *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) 3 months after application.

Figure 20. InocBloc™-treated girdling wounds on *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) 3 months after application.

Figure 21. Girdling wounds on *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) treated by grower practice (a spray with Nordox™) 3 months after application

3.4 Grafting

Statistical analysis of the qPCR results for the samples collected from the rootstock and scions before and after grafting showed no significant differences when all the data were analysed together (Table 14). However, examination of the incidence data (Figure 22) suggested that a discrete analysis of the rootstock samples collected 2 weeks after grafting was warranted. When those data were analysed separately, there were significant differences between treatments when the F3/R4 primers were used. Both the copper- and the InocBloc-treated samples had significantly (P=0.038) higher Ct values than the grower-practice samples (Table 14).

The average mean daily temperature during until 13 weeks was 11.4°C (Appendix 6).

Table 14. Mean *Pseudomonas syringae* pv. *actinidiae* (Psa) qPCR Crossing threshold (Ct) values for grafting wounds on *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) treated with copper and InocBloc™ when analysed using two primers F3/R4 and HopZ2b.

	F3/R4				HopZ2b		
	Copper	Grower practice	InocBloc	*P* value	Copper	Grower practice	InocBloc
Baseline rootstock	38.2±1.81	35.4±1.92	35.5±2.90		39.1±0.89	37.9±0.97	37.3±1.85
After 2 weeks scion	40	38.9±1.05	35.5±4.50		40	40	36.5±3.49
After 2 weeks rootstock*	37.9±2.06a	30.1±1.61b	37.4±2.52b	0.038	38.5±1.53	35.5±1.51	38.1±1.95
ANOVA				*P* value			
Treatment	0.148				0.369		
Time	0.318				0.553		
Treatment * Time	0.283				0.522		
Replicate	0.681				0.370		

*Numbers in a row with the same letter are not significantly different according to Tukey's test (α <0.05).

For samples taken 13 weeks after grafting from the rootstocks and the scion, there were fewer detections of Psa in grafts treated with InocBloc paste and copper paste than with the grower standard (petroleum jelly) (Figure 23), but the differences were not statistically significant.

After 26 weeks, on 19 December 2017, all the grafts that had been treated with copper paste were dead (Table 15). Two of five grafts treated with InocBloc were dead, and none of the grower standard grafts were dead (Table 15).

Table 15. Number of surviving *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) grafted on budwood 'Bruno' rootstocks in winter when assessed 26 weeks after application of wound protectants. Also recorded were number of fruit, any symptoms and the phenological growth stage (BBCH).

Treatment	Live	Dead	Symptoms	Number of fruit	BBCH
Copper paste	0	5	No growth, decay	0	0
InocBloc	3	2	Dieback on one vine	5, 4, 4	79
Grower standard	5	0	none	4, 6, 3, 4, 4	79

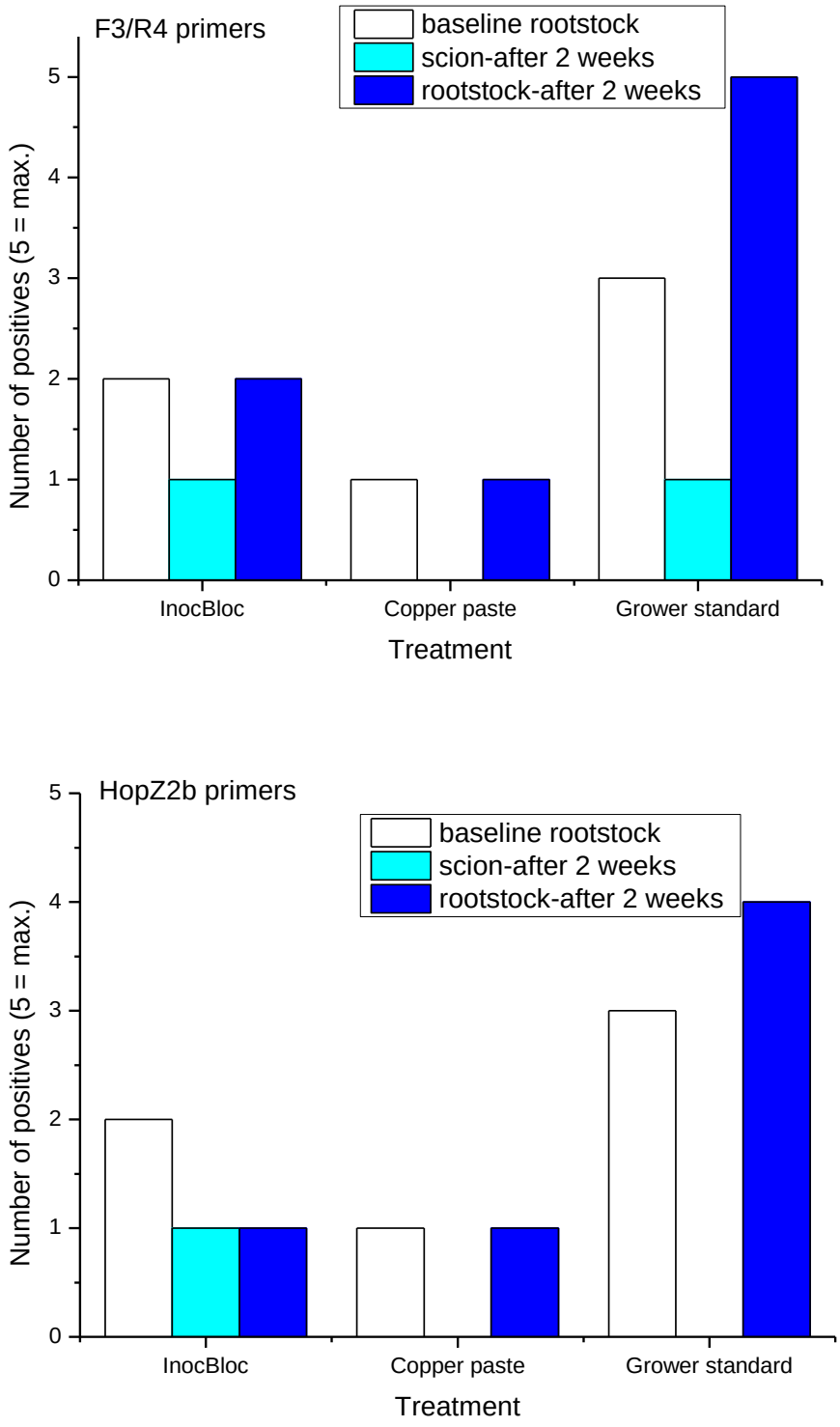

Figure 22. Number of *Pseudomonas syringae* pv. *actinidiae* (Psa)-positive samples by qPCR with F3/R4 (above) and HopZ2b (below) primers for samples taken at the same time as grafting (baseline) 'Bruno' rootstocks with *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) budwood in winter on 21 June 2017, and samples taken 2 weeks after application of wound protectants.

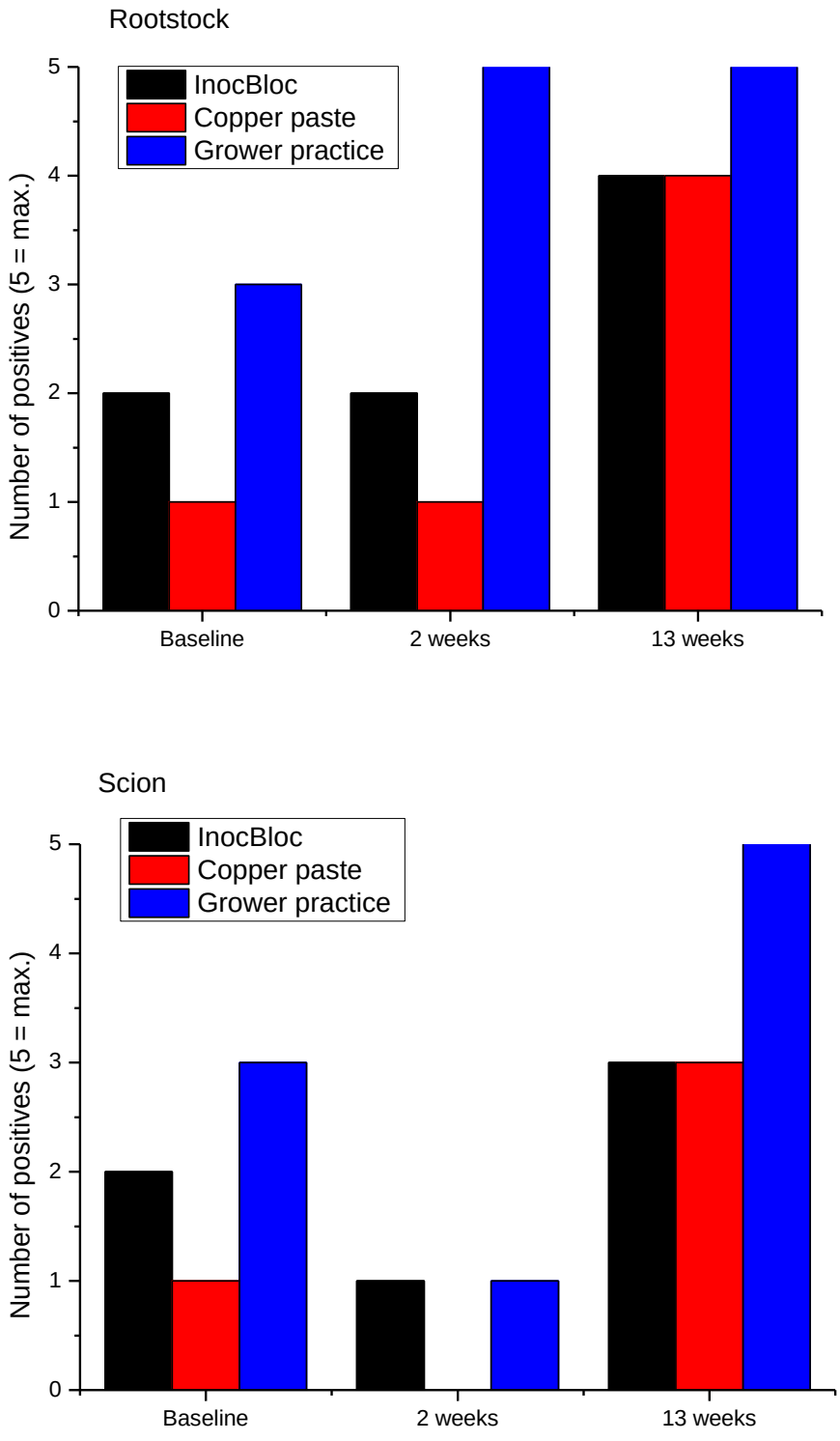

Figure 23. Number of *Pseudomonas syringae* pv. *actinidiae* (Psa)-positive samples by qPCR with F3/R4 primers for samples taken at the same time as grafting (baseline) 'Bruno' rootstocks with *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) budwood in winter, and samples taken 2 and 13 weeks after application of wound protectants. The baseline for the scion (lower) were samples taken from the rootstock (upper) at the time grafts were applied.

3.5 Temperature analysis

When the average mean daily temperature during the potted plant and mature vines experiments was plotted against the Ct values and incidence calculated using the F3/R4 primers for the inoculated and wounded controls (for the potted plant and mature vine experiments, respectively), there was a strong relationship (Figure 24). The R^2 was above 99% for both Gold3 and 'Hayward' plants, and the *P* value was <0.05. The relationship was not as strong when the HopZ2b primers were used (Figure 25), and three relationships were not statistically significant ('Hayward' Ct values and 'Hayward' and Gold3 incidence).

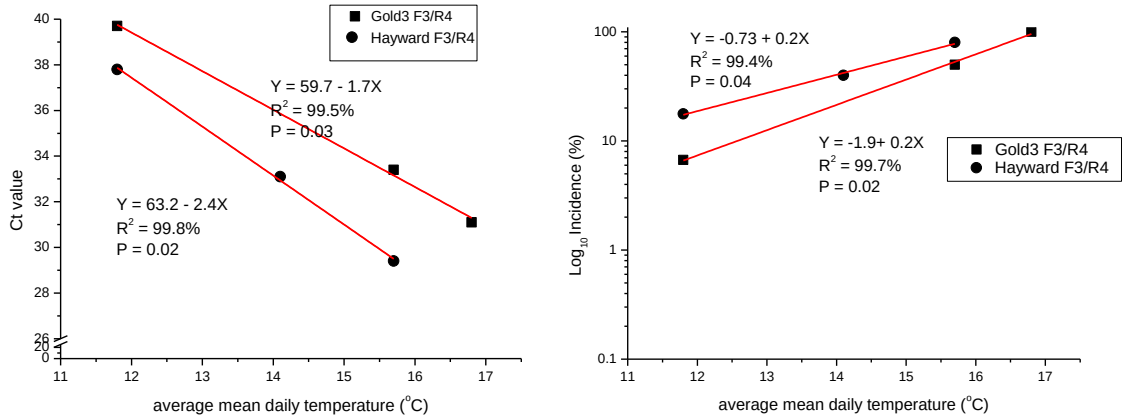

Figure 24. The relationship between Ct values (left) and incidence (right) of *Pseudomonas syringae* pv. *actinidiae* (Psa) detected by qPCR with F3/R4 primers and average mean daily temperature (°C). Results of wounded untreated and inoculated controls from wound protectant trials for *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) and *Actinidia chinensis* var. *deliciosa* 'Hayward' vines are presented.

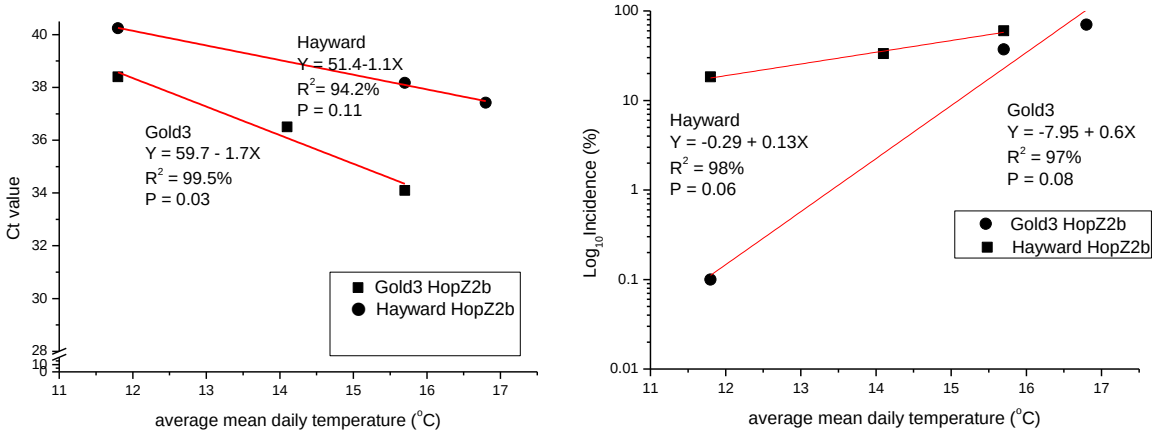

Figure 25. The relationship between Ct values (left) and incidence (right) of *Pseudomonas syringae* pv. *actinidiae* (Psa) detected by qPCR with HopZ2b primers and average mean daily temperature (°C). Results of wounded untreated and inoculated controls from wound protectant trials for *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) and *Actinidia chinensis* var. *deliciosa* 'Hayward' vines are presented.

Because there were no untreated controls in the girdling and grafting experiments, because comparisons were made with current grower practice, it was not possible to include these data points in the analysis. However, it is possible to use the predictive formulae derived from these analyses to predict the amount of Psa that would have occurred if the girdles and the grafts were not protected. For girdling that would have been 0.78% and for grafting 0.38% (calculated from the F3/R4 incidence formula from Figure 24; y = -1.9 + 0.2 x).

3.6 Phenological growth analysis

3.6.1 Gold3

There was a tendency for the copper paste treatment to result in more dead buds, and in longer dieback (Table 16). The differences in the distance of dieback were marginally statistically significant (*P*=0.06) following analysis of variance, but no treatments reduced the dieback compared with inoculated controls (Table 16). Analysis of the other phenological scores could not find any statistically significant differences between treatments, and it was difficult to interpret the results (Figure 26). However, there was a tendency for more dead buds for the wounds treated with copper paste, Damar spray and InocBloc paste (Figure 26). More development of flower buds was observed for wounds treated with Nordox, Topsin, New InocBloc paste and Damar biological (Figure 26). Raw results are presented in Appendix 7.

Table 16. Mean dieback distance (mm) and percent dead buds for *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) potted plants treated with wound protectants and assessed after 8 months in a standing out area in the orchard and sampled for PCR testing 16 weeks after treatments were applied.

Treatments	Dieback (mm)		% dead buds
1. Copper sulphate pentahydrate	139±34	a	40
2. Nordox™ 75WG	54±16.6	a	10
3. Greenseal™ Ultra	47±12	ab	10
4. Topsin® M-4A	54±15	ab	0
5. Damar® biological	45±15.6	ab	30
6. InocBloc™ new formulation	34±4.3	b	0
7. InocBloc paste	45±15.6	ab	30
8. uninoculated control	59±31	ab	10
9. inoculated control	79±27	ab	10
ANOVA *P* value		0.06	

* Numbers in a column with the same letter are not significantly different according to Tukey's test (α <0.05).

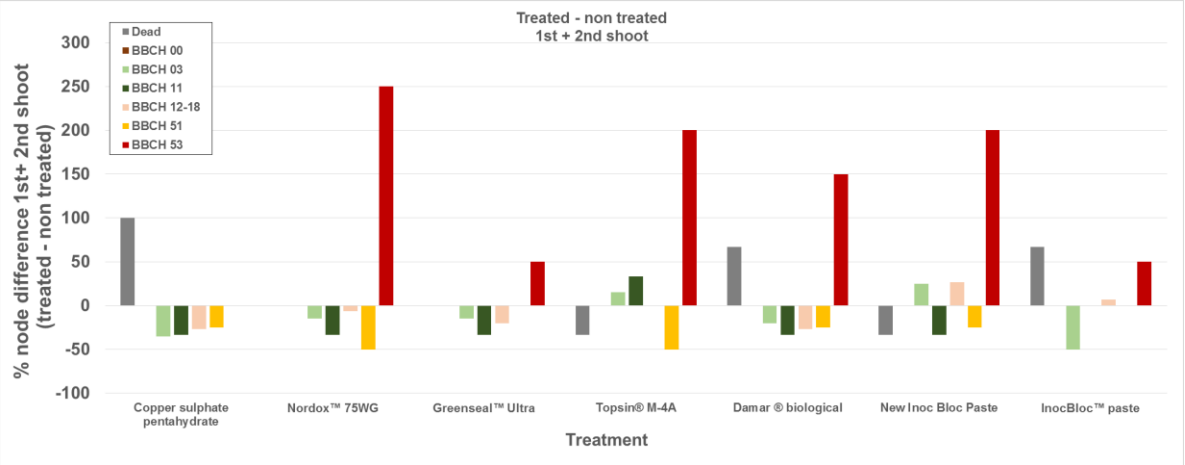

Figure 26. The difference in BBCH (Biologische Bundnesanstalt, Bundessortenamt and Chemical industry) scores between untreated and treated for *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) potted plants treated with wound protectants, then inoculated with *Pseudomonas syringae* pv. *actinidiae* and assessed after 8 months in a standing out area in the orchard. A positive value indicates worse (for dead) or better (for all other scores) than controls. These replicates were sampled for PCR 16 weeks after treatments were applied.

3.6.2 'Hayward'

When the replicates that had not been sampled for PCR were analysed, there was a tendency for the copper paste treatment to result in more dead buds, and in longer dieback (Table 17). The differences in the distance of dieback were not statistically significant (P=0.76) following analysis of variance. Dieback was less than inoculated controls for wounds treated with both formulations of InocBloc, Greenseal and Topsin (Table 17). Analysis of the other phenological scores could not find any statistically significant differences between treatments, and it was difficult to interpret the results (Figure 27). However, there was a tendency for more development of leaves for the wounds treated with copper paste, Damar spray and InocBloc paste (Figure 27). Raw results are presented in Appendix 8.

Table 17. Mean dieback distance (mm) and percent dead buds for *A. chinensis* var. *deliciosa* 'Hayward' potted plants treated with wound protectants and assessed after 3 months in a standing out area in the orchard, and not sampled for PCR testing.

Treatments	Dieback (mm)	% dead buds
1. Copper sulphate pentahydrate	73.8±21	20
2. Nordox™ 75WG	26.6±17.9	20
3. Greenseal™ Ultra	17±10.5	0
4. Topsin® M-4A	22.4±14.4	0
5. Damar® biological	65±58.8	20
6. InocBloc™ new formulation	13±7.6	0
7. InocBloc paste	16.8±10.9	0
8. uninoculated control	9.2±7.7	0
9. inoculated control	27±19.3	20
ANOVA *P* value	0.76	

* Numbers in a column with the same letter are not significantly different according to Tukey's test (α <0.05).

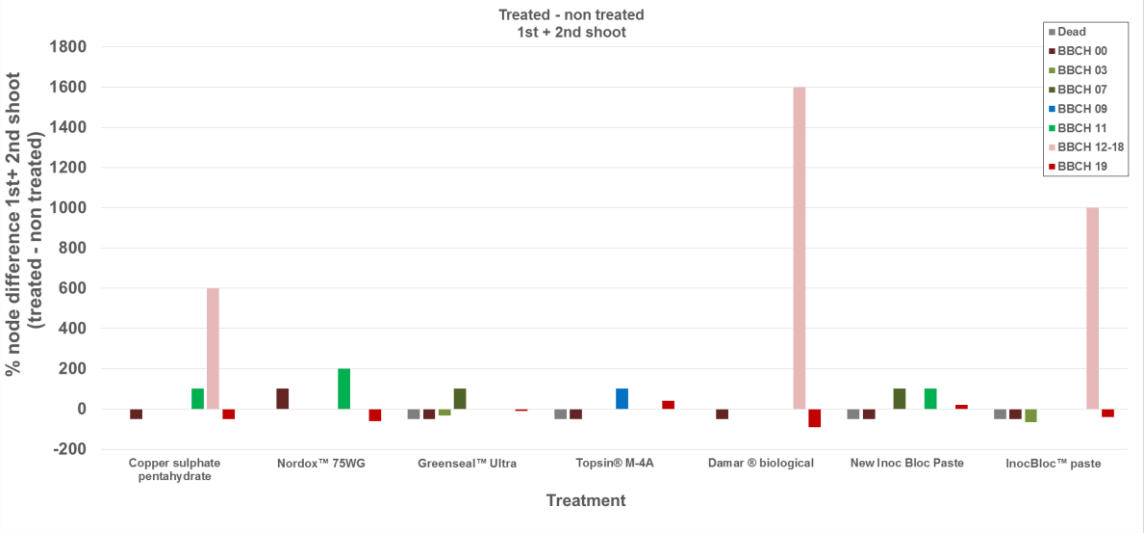

Figure 27. The difference in BBCH (Biologische Bundesanstalt, Bundessortenamt and Chemical industry) scores between untreated and treated for *A. chinensis* var. *deliciosa* 'Hayward' potted plants treated with wound protectants, then inoculated with *Pseudomonas syringae* pv. *actinidiae* and assessed after 3 months in a standing out area in the orchard. A positive value indicates worse (for dead) or better (for all other scores) than untreated inoculated controls. These replicates were not sampled for PCR testing.

When the replicates that had been sampled for PCR were analysed, there was a tendency for the Damar spray treatment to result in more dead buds, followed by InocBloc, copper paste and InocBloc new formulation (Table 18). The differences in the distance of dieback were not statistically significant (*P*=0.93) following analysis of variance. Dieback was less than inoculated controls for wounds treated with the new formulation of InocBloc, Nordox, copper paste, Greenseal and Topsin (Table 18). Analysis of the other phenological scores could not find any statistically significant differences between treatments, and it was difficult to interpret the results (Figure 28). Raw results are presented in Appendix 9.

Table 18. Mean dieback distance (mm) and percent dead buds for *A. chinensis* var. *deliciosa* 'Hayward' potted plants treated with wound protectants and assessed after 3 months in a standing out area in the orchard, and sampled for PCR testing 6 weeks after treatments were applied.

Treatments	Dieback (mm)	% dead buds
1. Copper sulphate pentahydrate	309.4±195.6	50
2. Nordox™ 75WG	307.6±131.6	0
3. Greenseal™ Ultra	258.6±167.2	0
4. Topsin® M-4A	264.2±259	0
5. Damar ® biological	334.6±204.3	80
6. InocBloc™ new formulation	284.4±173.9	50
7. InocBloc paste	364.2±223.4	60
8. uninoculated control	144.8±143.8	0
9. inoculated control	329.4±181.4	80
ANOVA *P* value	0.93	

* Numbers in a column with the same letter are not significantly different according to Tukey's test (α <0.05).

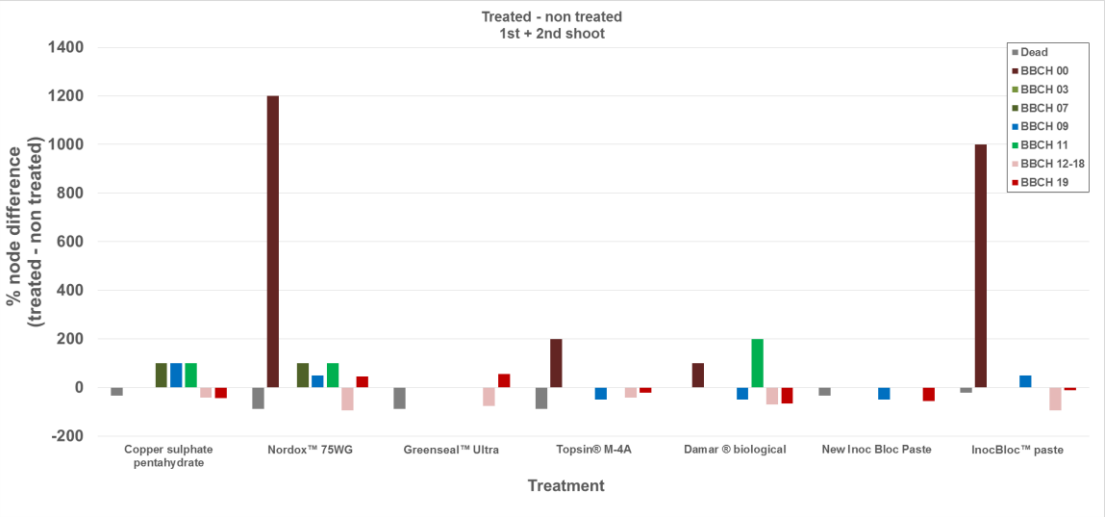

Figure 28. The difference in BBCH (Biologische Bundesanstalt, Bundessortenamt and Chemical industry) scores between untreated and treated for *A. chinensis* var. *deliciosa* 'Hayward' potted plants treated with wound protectants, then inoculated with *Pseudomonas syringae* pv. *actinidiae* and assessed after 3 months in a standing out area in the orchard. A positive value indicates worse (for dead) or better (for all other scores) than untreated inoculated controls. These replicates were sampled for PCR 6 weeks after treatments were applied.

4 DISCUSSION

Results from both Gold3 and 'Hayward' potted plant experiments and spring pruning trials showed that Topsin and Greenseal were ineffective wound protectants.

There were some differences in the effectiveness of the best protectants between the two potted plant experiments, possibly because the method of inoculation was different. Inoculum was sprayed onto wounds in the Gold3 experiment, and aliquoted onto wounds with a pipette in the 'Hayward' experiment. For spray inoculations on Gold3, InocBloc paste and new formulation and both copper paste and Nordox spray were the most effective protectants, whereas Greenseal, Topsin and Damar biological were the least effective. In contrast, on 'Hayward' potted plants, copper paste and Nordox spray were the least effective, whilst Damar biological gave some efficacy. InocBloc paste and new formulation were the most effective wound protectants on 'Hayward' potted plants.

However, when the BBCH scores were examined, there may have been an adverse effect on plant growth by copper paste. None of the parameters measured gave statistically significant differences, but application of copper paste resulted in more dieback than all other treatments for Gold3 and for 'Hayward' that was not sampled for PCR. In previous work it was shown that dieback was not related to the incidence of Psa, and was probably an indication of an effective plant response to infection (Everett et al. 2014).

The BBCH scores were difficult to interpret. It was confounded by the difference in plant ages for the 'Hayward' assessments, which is why both the replicates sampled for PCR and the replicates that had remained intact were assessed separately. There was a difference in the number of plants that died between the two sets of replicates. Further work examining the phenology of plants for which the wounds are treated with the most promising wound protectants (copper paste, InocBloc (both formulations) and Nordox) without re-wounding before assessments, and without differences in ages, may yield results that are easier to interpret.

Natural infections were too low in the winter pruning trial to test the effectiveness of wound protectants.

There was sufficient natural inoculum for testing wound protectants in the spring pruning trial. On both 'Hayward' and Gold3, copper paste was the most effective treatment, followed by the two InocBloc formulations. In spring, a spray with Nordox was not effective, nor were Topsin, Damar biological or Greenseal.

Incidence of Psa detected in the girdling trial was low in late February and through the winter, but increased in spring. The girdle wounds were clearly not protected by copper paste, and not sufficiently healed after application of InocBloc paste to protect the wounds. The current grower practice was sufficient to prevent infections of the girdling wounds, and to allow fast healing of the wounds. Callus formation was not obviously inhibited by the application of Nordox to the girdling wounds (grower practice).

The results of the grafting trial suggest that copper paste should not be used, and that InocBloc paste was not as effective as the current grower practice.

Our results suggest that in spring, when temperatures averaged 15.7°C, resampling treatments after 2–3 weeks was sufficient to detect differences between treatments, but when temperatures are lower during winter, more time will be needed after application of treatments to detect differences. A strong inverse correlation was found between temperature and incidence of Psa, which suggested that during winter there was insufficient inoculum available to infect plants.

It is possible that the high mortality after 26 weeks for the vines treated with copper paste in the girdling and grafting trials was because the wounds did not heal, and these vines were infected by Psa once temperatures rose and inoculum became available in spring, although inoculum was not measured. The mortality of some grafts treated with InocBloc paste indicates that these wounds probably did not heal as well as when the grafts were coated with petroleum jelly, of which none died.

Overall, InocBloc paste and it's new formulation, along with copper paste, were the most consistent effective wound protectants when used on pruning wounds. However, they should not be applied to girdling wounds or grafting sites. Application of this concentrated form of copper appeared to be the cause of death of two out of five girdled vines, and five out of five grafts. Deaths of girdled vines were not caused by InocBloc, but overcallusing resulted in cracking which may have allowed entry of Psa. Two out of five grafts were killed following application of InocBloc.

5 REFERENCES

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APPENDICES

Appendix 1. Te Puke Research Centre weather during Gold3 potted plant trials, and the girdling trial

Mean, maximum and minimum daily temperatures (°C) and daily rainfall (mm) at Te Puke Research Centre, Bay of Plenty, during *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) potted plant trials from 2 February to 26 May 2017.

Start date	Stop date	Rain total	Temperature dry bulb mean	Temperature dry bulb min	Temperature dry bulb max
1/02/2017	2/02/2017	0	20.2	16.5	25.4
2/02/2017	3/02/2017	0.2	20.9	18	24.7
3/02/2017	4/02/2017	0	19.4	10.1	28.2
4/02/2017	5/02/2017	0	20.2	14.5	26.6
5/02/2017	6/02/2017	0	19	12.7	25.4
6/02/2017	7/02/2017	0	21.9	17.2	28.9
7/02/2017	8/02/2017	0	23.8	20.2	29.4
8/02/2017	9/02/2017	4	17	14.1	21.8
9/02/2017	10/02/2017	0.2	15.3	9.8	23
10/02/2017	11/02/2017	0	15.9	10.3	21.3
11/02/2017	12/02/2017	0	17.4	11.9	23.6
12/02/2017	13/02/2017	1.2	20.3	15.9	23.7
13/02/2017	14/02/2017	0	21.4	17.5	26.7
14/02/2017	15/02/2017	0	18.8	15.2	22
15/02/2017	16/02/2017	4.6	20.7	16	27.1
16/02/2017	17/02/2017	112.4	18.5	16.5	19.3
17/02/2017	18/02/2017	64.6	19	17.6	22.6
18/02/2017	19/02/2017	19	20.3	18.8	23
19/02/2017	20/02/2017	8.8	20.5	17.7	24.2
20/02/2017	21/02/2017	0.2	18.7	15.7	22.8
21/02/2017	22/02/2017	0	18.1	14.1	22.8
22/02/2017	23/02/2017	0	19.7	16	23.9
23/02/2017	24/02/2017	0	19.4	14.5	24.4
24/02/2017	25/02/2017	0	19.2	15.2	24.3
25/02/2017	26/02/2017	0	19.1	14.6	25.2
26/02/2017	27/02/2017	0	20.5	15.7	26.1
27/02/2017	28/02/2017	0	19.3	17.2	21.4
28/02/2017	1/03/2017	0	20.1	17.9	23.8
1/03/2017	2/03/2017	0	19.6	15.5	23.9
2/03/2017	3/03/2017	0	19	13	25.2
3/03/2017	4/03/2017	0	18.6	13.1	24.8
4/03/2017	5/03/2017	0	18.8	11.1	25.1
5/03/2017	6/03/2017	0	17.7	11.9	23.8
6/03/2017	7/03/2017	0	18.1	12.5	25

Start date	Stop date	Rain total	Temperature dry bulb mean	Temperature dry bulb min	Temperature dry bulb max
7/03/2017	8/03/2017	85	16.8	12.7	22
8/03/2017	9/03/2017	29.2	13.5	12.8	14.6
9/03/2017	10/03/2017	0.6	17.4	14.6	19.8
10/03/2017	11/03/2017	85.4	20.5	17.8	22.8
11/03/2017	12/03/2017	19.4	20.4	19.8	21.1
12/03/2017	13/03/2017	18.8	19.4	17.3	22.5
13/03/2017	14/03/2017	0	17.5	11.4	24.5
14/03/2017	15/03/2017	0	16.9	12.5	22.5
15/03/2017	16/03/2017	0	15.4	10.9	21.1
16/03/2017	17/03/2017	0	16	10.3	22.8
17/03/2017	18/03/2017	0	17.7	13.3	22.5
18/03/2017	19/03/2017	0	17.7	12	24.1
19/03/2017	20/03/2017	0	16	10.6	22.6
20/03/2017	21/03/2017	0	17	12.5	22.3
21/03/2017	22/03/2017	0	19.3	14.3	26.5
22/03/2017	23/03/2017	0	20	17.5	23.4
23/03/2017	24/03/2017	2.6	21.7	5.2	27.3
24/03/2017	25/03/2017	0	19.4	14.6	24.5
25/03/2017	26/03/2017	2.6	20.1	17.3	25
26/03/2017	27/03/2017	42.2	20.5	18.7	23.5
27/03/2017	28/03/2017	9	20.5	17.4	24.1
28/03/2017	29/03/2017	37	20.8	17.8	25.2
29/03/2017	30/03/2017	21	18.7	17.8	20.4
30/03/2017	31/03/2017	0	19	12.3	25
31/03/2017	1/04/2017	0	19.1	14.5	26.4
1/04/2017	2/04/2017	0	18.4	14.7	24
2/04/2017	3/04/2017	3.8	20.9	17.1	24.5
3/04/2017	4/04/2017	27	21.7	17.4	26
4/04/2017	5/04/2017	182.8	16	14.5	19.4
5/04/2017	6/04/2017	75.4	18.7	14.2	21.4
6/04/2017	7/04/2017	1	16.2	11.3	21.4
7/04/2017	8/04/2017	0	14.8	7.2	21.9
8/04/2017	9/04/2017	0	14.1	8.1	21.7
9/04/2017	10/04/2017	0	15.8	12.6	21
10/04/2017	11/04/2017	0.2	18.4	15.6	22.7
11/04/2017	12/04/2017	12	19.8	18.6	21.9
12/04/2017	13/04/2017	47.6	19.3	17.7	20.2
13/04/2017	14/04/2017	85	17.1	15	20.1
14/04/2017	15/04/2017	0	16.8	12.4	22.6
15/04/2017	16/04/2017	0.6	15.2	10.3	22.2
16/04/2017	17/04/2017	9.6	15.4	11	22.1
17/04/2017	18/04/2017	0.8	14.6	11.4	19.6
18/04/2017	19/04/2017	0	15.6	11.4	21.9

Start date	Stop date	Rain total	Temperature dry bulb mean	Temperature dry bulb min	Temperature dry bulb max
19/04/2017	20/04/2017	0	14.8	8.9	21.5
20/04/2017	21/04/2017	0	13	5.9	21.1
21/04/2017	22/04/2017	0	12.7	8.2	19.5
22/04/2017	23/04/2017	0	14.1	10	20.9
23/04/2017	24/04/2017	0	15.8	13.5	19.4
24/04/2017	25/04/2017	0	15.9	11.3	22.3
25/04/2017	26/04/2017	0	15	8.4	23.1
26/04/2017	27/04/2017	0	14.6	8.9	22.2
27/04/2017	28/04/2017	0	14.2	7.7	22.5
28/04/2017	29/04/2017	0	16.4	12.9	21.7
29/04/2017	30/04/2017	27.8	18.1	16.7	18.8
30/04/2017	1/05/2017	3.6	14.2	8.6	20
1/05/2017	2/05/2017	0	10.6	5.2	16.4
2/05/2017	3/05/2017	0	11.2	4.8	18.2
3/05/2017	4/05/2017	1.2	15.1	11.7	18.8
4/05/2017	5/05/2017	0	12	6.9	17.6
5/05/2017	6/05/2017	0	11.4	5.3	19.5
6/05/2017	7/05/2017	0	12.2	6.8	20.2
7/05/2017	8/05/2017	0	13	8.3	19.4
8/05/2017	9/05/2017	0	11.9	7.7	19
9/05/2017	10/05/2017	0	14.3	10.7	20.4
10/05/2017	11/05/2017	5.2	16.8	14.3	20.9
11/05/2017	12/05/2017	58.8	18.5	16.6	19.3
12/05/2017	13/05/2017	37.8	13.8	10.7	19
13/05/2017	14/05/2017	0	9.6	3.9	16
14/05/2017	15/05/2017	0	10.4	5	17.4
15/05/2017	16/05/2017	0	11.8	6.7	18.3
16/05/2017	17/05/2017	4.2	14.6	11.4	16.8
17/05/2017	18/05/2017	51.4	15.8	12.6	17.7
18/05/2017	19/05/2017	5	15.1	12.2	18.8
19/05/2017	20/05/2017	0	12.9	10.1	16.4
20/05/2017	21/05/2017	2.8	7.3	0.9	15.7
21/05/2017	22/05/2017	0	6.7	0.9	13.8
22/05/2017	23/05/2017	0	6	0.5	15
23/05/2017	24/05/2017	0	11.3	7.1	14.4
24/05/2017	25/05/2017	6.6	11.7	7.4	15.9

Appendix 2. Te Puke Research Centre weather during 'Hayward' potted plant trials

Mean, maximum and minimum daily temperatures (°C) and daily rainfall (mm) at Te Puke Research Centre, Bay of Plenty, during *Actinidia chinensis* var. *deliciosa* 'Hayward' potted plant trials from 4 October to 15 November 2017.

Start date	Stop date	Rain total	Temperature dry bulb mean	Temperature dry bulb min	Temperature dry bulb max
3/10/2017	4/10/2017	0	13.6	8.5	19.4
4/10/2017	5/10/2017	0	14.7	11.5	19.6
5/10/2017	6/10/2017	4.2	14.1	10.9	21.4
6/10/2017	7/10/2017	0	13.3	11.3	16.4
7/10/2017	8/10/2017	24	14.5	13.4	16.1
8/10/2017	9/10/2017	66.2	13.3	12.4	14.1
9/10/2017	10/10/2017	31.2	13.8	12.2	16.8
10/10/2017	11/10/2017	5.2	14.8	11.7	18.9
11/10/2017	12/10/2017	0	13	9.7	17.5
12/10/2017	13/10/2017	0	13.4	7	19.6
13/10/2017	14/10/2017	0	14.3	11	17.4
14/10/2017	15/10/2017	0	14.5	7.4	21.8
15/10/2017	16/10/2017	0	13.2	7.7	17.7
16/10/2017	17/10/2017	0	14	6.4	20.3
17/10/2017	18/10/2017	0	13.8	9.6	19.3
18/10/2017	19/10/2017	0.2	12.4	5.1	20.5
19/10/2017	20/10/2017	0	11.8	6.9	16.9
20/10/2017	21/10/2017	0	14.8	12.8	18.6
21/10/2017	22/10/2017	0	15.4	13.5	19.9
22/10/2017	23/10/2017	1.6	13.6	9.5	19.1
23/10/2017	24/10/2017	7.8	11.8	6.7	15.1
24/10/2017	25/10/2017	0	14.2	7.3	20.7
25/10/2017	26/10/2017	0.6	14.7	12	19.2
26/10/2017	27/10/2017	9.2	16.5	12.3	22.3
27/10/2017	28/10/2017	15	13	12.1	14.2
28/10/2017	29/10/2017	47.4	13.6	12.3	14.1
29/10/2017	30/10/2017	2.6	14	11.3	16.2
30/10/2017	31/10/2017	0	14.9	11.9	19.3
31/10/2017	1/11/2017	1.4	14.7	12.2	19.4
1/11/2017	2/11/2017	0	15.3	13.2	18.6
2/11/2017	3/11/2017	1.2	15.1	12.9	17.8
3/11/2017	4/11/2017	0.6	16.2	15.7	17.4
4/11/2017	5/11/2017	0.8	16.1	11.4	19.8
5/11/2017	6/11/2017	1.8	15.6	11.2	20.6
6/11/2017	7/11/2017	0	13.1	5.7	19.9
7/11/2017	8/11/2017	16	16	13.1	17.9
8/11/2017	9/11/2017	1.8	12.7	4.7	19.1

Start date	Stop date	Rain total	Temperature dry bulb mean	Temperature dry bulb min	Temperature dry bulb max
9/11/2017	10/11/2017	0	14.5	8.1	20.4
10/11/2017	11/11/2017	0	14.9	11.2	21
11/11/2017	12/11/2017	0.4	12.7	10.8	15.8
12/11/2017	13/11/2017	0	13.1	6.8	18.2
13/11/2017	14/11/2017	0.6	13.8	9.9	19.3
14/11/2017	15/11/2017	7.8	13.5	9.9	18
15/11/2017	16/11/2017	0	14.1	11.1	18.1

Appendix 3. Te Puke Research Centre weather during winter pruning trials

Mean, maximum and minimum daily temperatures (°C) and daily rainfall (mm) at Te Puke Research Centre, Bay of Plenty, during *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) and *A. chinensis* var. *deliciosa* 'Hayward' winter pruning mature plant trials from 3 August to 18 August 2017.

Start date	Stop date	Rain total	Temperature dry bulb mean	Temperature dry bulb min	Temperature dry bulb max
3/08/2017	4/08/2017	0	8.3	2.9	15.8
4/08/2017	5/08/2017	0	8	4.4	14.3
5/08/2017	6/08/2017	0	8.3	3.3	16.2
6/08/2017	7/08/2017	17.4	13	6.9	14.4
7/08/2017	8/08/2017	0	13.5	9.9	20
8/08/2017	9/08/2017	12.4	14.6	12.7	15.7
9/08/2017	10/08/2017	27.8	13.4	8.3	15.6
10/08/2017	11/08/2017	1.2	10.7	5.9	17.1
11/08/2017	12/08/2017	0	13.1	11	16.6
12/08/2017	13/08/2017	0	14.1	9.8	19.2
13/08/2017	14/08/2017	0	13.4	7.9	18.7
14/08/2017	15/08/2017	1	10.5	4.6	17.2
15/08/2017	16/08/2017	0.4	10.2	5.8	15.8
16/08/2017	17/08/2017	0.6	11.4	7.5	15.9
17/08/2017	18/08/2017	0.4	12.7	6.9	17.5
18/08/2017	19/08/2017	19.2	13.6	11.1	18.4

Appendix 4. Te Puke Research Centre weather during spring pruning trials

Mean, maximum and minimum daily temperatures (°C) and daily rainfall (mm) at Te Puke Research Centre, Bay of Plenty, during *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) and *A. chinensis* var. *deliciosa* 'Hayward' spring pruning mature plant trials from 8 to 29 November 2017.

Start date	Stop date	Rain total	Temperature dry bulb mean	Temperature dry bulb min	Temperature dry bulb max
7/11/2017	8/11/2017	16	16	13.1	17.9
8/11/2017	9/11/2017	1.8	12.7	4.7	19.1
9/11/2017	10/11/2017	0	14.5	8.1	20.4
10/11/2017	11/11/2017	0	14.9	11.2	21
11/11/2017	12/11/2017	0.4	12.7	10.8	15.8
12/11/2017	13/11/2017	0	13.1	6.8	18.2
13/11/2017	14/11/2017	0.6	13.8	9.9	19.3
14/11/2017	15/11/2017	7.8	13.5	9.9	18
15/11/2017	16/11/2017	0	14.1	11.1	18.1
16/11/2017	17/11/2017	0	13.2	8.8	18.6
17/11/2017	18/11/2017	0	16.3	11.9	21.2
18/11/2017	19/11/2017	2.4	15.4	12.4	20.1
19/11/2017	20/11/2017	0.2	15.6	12.7	20
20/11/2017	21/11/2017	0	15.4	7.7	23.6
21/11/2017	22/11/2017	0	15.3	10.7	20.1
22/11/2017	23/11/2017	0	16.8	12.3	21
23/11/2017	24/11/2017	0	18.7	15.2	23.1
24/11/2017	25/11/2017	0	18.9	16.7	22.5
25/11/2017	26/11/2017	0	17.9	15.7	21.7
26/11/2017	27/11/2017	0	17.7	15.7	21.7
27/11/2017	28/11/2017	0	18.2	15.9	21.8
28/11/2017	29/11/2017	0	17.5	13.5	21.5
29/11/2017	30/11/2017	0	18.5	15.6	22.7

Appendix 5. Te Puke Research Centre weather data during the girdling trial

Mean, maximum and minimum daily temperatures (°C) and daily rainfall (mm) at Te Puke Research Centre, Bay of Plenty, during the girdling trial from 23 February to 14 September 2017. The trial was conducted on a grower property c. 5 km towards the coast from the Te Puke Research Centre.

Start date	Stop date	Rain total	Temperature dry bulb mean	Temperature DRY BULB MIN	Temperature dry bulb max
22/02/2017	23/02/2017	0	19.7	16	23.9
23/02/2017	24/02/2017	0	19.4	14.5	24.4
24/02/2017	25/02/2017	0	19.2	15.2	24.3
25/02/2017	26/02/2017	0	19.1	14.6	25.2
26/02/2017	27/02/2017	0	20.5	15.7	26.1
27/02/2017	28/02/2017	0	19.3	17.2	21.4
28/02/2017	1/03/2017	0	20.1	17.9	23.8
1/03/2017	2/03/2017	0	19.6	15.5	23.9
2/03/2017	3/03/2017	0	19	13	25.2
3/03/2017	4/03/2017	0	18.6	13.1	24.8
4/03/2017	5/03/2017	0	18.8	11.1	25.1
5/03/2017	6/03/2017	0	17.7	11.9	23.8
6/03/2017	7/03/2017	0	18.1	12.5	25
7/03/2017	8/03/2017	85	16.8	12.7	22
8/03/2017	9/03/2017	29.2	13.5	12.8	14.6
9/03/2017	10/03/2017	0.6	17.4	14.6	19.8
10/03/2017	11/03/2017	85.4	20.5	17.8	22.8
11/03/2017	12/03/2017	19.4	20.4	19.8	21.1
12/03/2017	13/03/2017	18.8	19.4	17.3	22.5
13/03/2017	14/03/2017	0	17.5	11.4	24.5
14/03/2017	15/03/2017	0	16.9	12.5	22.5
15/03/2017	16/03/2017	0	15.4	10.9	21.1
16/03/2017	17/03/2017	0	16	10.3	22.8
17/03/2017	18/03/2017	0	17.7	13.3	22.5
18/03/2017	19/03/2017	0	17.7	12	24.1
19/03/2017	20/03/2017	0	16	10.6	22.6
20/03/2017	21/03/2017	0	17	12.5	22.3
21/03/2017	22/03/2017	0	19.3	14.3	26.5
22/03/2017	23/03/2017	0	20	17.5	23.4
23/03/2017	24/03/2017	2.6	21.7	5.2	27.3
24/03/2017	25/03/2017	0	19.4	14.6	24.5
25/03/2017	26/03/2017	2.6	20.1	17.3	25
26/03/2017	27/03/2017	42.2	20.5	18.7	23.5
27/03/2017	28/03/2017	9	20.5	17.4	24.1
28/03/2017	29/03/2017	37	20.8	17.8	25.2
29/03/2017	30/03/2017	21	18.7	17.8	20.4

Start date	Stop date	Rain total	Temperature dry bulb mean	Temperature DRY BULB MIN	Temperature dry bulb max
30/03/2017	31/03/2017	0	19	12.3	25
31/03/2017	1/04/2017	0	19.1	14.5	26.4
1/04/2017	2/04/2017	0	18.4	14.7	24
2/04/2017	3/04/2017	3.8	20.9	17.1	24.5
3/04/2017	4/04/2017	27	21.7	17.4	26
4/04/2017	5/04/2017	182.8	16	14.5	19.4
5/04/2017	6/04/2017	75.4	18.7	14.2	21.4
6/04/2017	7/04/2017	1	16.2	11.3	21.4
7/04/2017	8/04/2017	0	14.8	7.2	21.9
8/04/2017	9/04/2017	0	14.1	8.1	21.7
9/04/2017	10/04/2017	0	15.8	12.6	21
10/04/2017	11/04/2017	0.2	18.4	15.6	22.7
11/04/2017	12/04/2017	12	19.8	18.6	21.9
12/04/2017	13/04/2017	47.6	19.3	17.7	20.2
13/04/2017	14/04/2017	85	17.1	15	20.1
14/04/2017	15/04/2017	0	16.8	12.4	22.6
15/04/2017	16/04/2017	0.6	15.2	10.3	22.2
16/04/2017	17/04/2017	9.6	15.4	11	22.1
17/04/2017	18/04/2017	0.8	14.6	11.4	19.6
18/04/2017	19/04/2017	0	15.6	11.4	21.9
19/04/2017	20/04/2017	0	14.8	8.9	21.5
20/04/2017	21/04/2017	0	13	5.9	21.1
21/04/2017	22/04/2017	0	12.7	8.2	19.5
22/04/2017	23/04/2017	0	14.1	10	20.9
23/04/2017	24/04/2017	0	15.8	13.5	19.4
24/04/2017	25/04/2017	0	15.9	11.3	22.3
25/04/2017	26/04/2017	0	15	8.4	23.1
26/04/2017	27/04/2017	0	14.6	8.9	22.2
27/04/2017	28/04/2017	0	14.2	7.7	22.5
28/04/2017	29/04/2017	0	16.4	12.9	21.7
29/04/2017	30/04/2017	27.8	18.1	16.7	18.8
30/04/2017	1/05/2017	3.6	14.2	8.6	20
1/05/2017	2/05/2017	0	10.6	5.2	16.4
2/05/2017	3/05/2017	0	11.2	4.8	18.2
3/05/2017	4/05/2017	1.2	15.1	11.7	18.8
4/05/2017	5/05/2017	0	12	6.9	17.6
5/05/2017	6/05/2017	0	11.4	5.3	19.5
6/05/2017	7/05/2017	0	12.2	6.8	20.2
7/05/2017	8/05/2017	0	13	8.3	19.4
8/05/2017	9/05/2017	0	11.9	7.7	19
9/05/2017	10/05/2017	0	14.3	10.7	20.4
10/05/2017	11/05/2017	5.2	16.8	14.3	20.9
11/05/2017	12/05/2017	58.8	18.5	16.6	19.3

Start date	Stop date	Rain total	Temperature dry bulb mean	Temperature DRY BULB MIN	Temperature dry bulb max
12/05/2017	13/05/2017	37.8	13.8	10.7	19
13/05/2017	14/05/2017	0	9.6	3.9	16
14/05/2017	15/05/2017	0	10.4	5	17.4
15/05/2017	16/05/2017	0	11.8	6.7	18.3
16/05/2017	17/05/2017	4.2	14.6	11.4	16.8
17/05/2017	18/05/2017	51.4	15.8	12.6	17.7
18/05/2017	19/05/2017	5	15.1	12.2	18.8
19/05/2017	20/05/2017	0	12.9	10.1	16.4
20/05/2017	21/05/2017	2.8	7.3	0.9	15.7
21/05/2017	22/05/2017	0	6.7	0.9	13.8
22/05/2017	23/05/2017	0	6	0.5	15
23/05/2017	24/05/2017	0	11.3	7.1	14.4
24/05/2017	25/05/2017	6.6	11.7	7.4	15.9
25/05/2017	26/05/2017	0	11.1	6.7	18.4
26/05/2017	27/05/2017	44.8	15.7	11.6	17.6
27/05/2017	28/05/2017	27	14.9	13.7	16.2
28/05/2017	29/05/2017	0	13.7	11.3	19.3
29/05/2017	30/05/2017	0	12.5	7.2	20.2
30/05/2017	31/05/2017	0.2	12.6	10.4	17.9
31/05/2017	1/06/2017	0	12.1	7.5	19.6
1/06/2017	2/06/2017	0	11.6	7.7	18.6
2/06/2017	3/06/2017	0	13.3	10.7	17.9
3/06/2017	4/06/2017	0	10.9	6.3	17.2
4/06/2017	5/06/2017	0	9.4	4.3	16.8
5/06/2017	6/06/2017	9.2	10.6	7	15.4
6/06/2017	7/06/2017	0	8.6	3.5	15.7
7/06/2017	8/06/2017	0	8.9	3.4	16.1
8/06/2017	9/06/2017	0	8	1.6	15
9/06/2017	10/06/2017	0	10	4.8	16.9
10/06/2017	11/06/2017	0	10.2	4	17.7
11/06/2017	12/06/2017	0	10	3.6	17.5
12/06/2017	13/06/2017	0	11.5	7.2	16.2
13/06/2017	14/06/2017	0.4	14.2	10.9	17.4
14/06/2017	15/06/2017	0.2	8	0.3	15.6
15/06/2017	16/06/2017	0	11.4	4.2	15.1
16/06/2017	17/06/2017	0	13	9.6	18.2
17/06/2017	18/06/2017	12.6	12.4	11.8	13.5
18/06/2017	19/06/2017	2	11.5	8.8	14.1
19/06/2017	20/06/2017	0	10.6	6.8	16.2
20/06/2017	21/06/2017	0	12	7.6	18.1
21/06/2017	22/06/2017	10.8	13.4	11.8	15
22/06/2017	23/06/2017	49.6	14.4	13.4	16.4
23/06/2017	24/06/2017	25.2	15.7	13.3	17.4

Start date	Stop date	Rain total	Temperature dry bulb mean	Temperature DRY BULB MIN	Temperature dry bulb max
24/06/2017	25/06/2017	0	11.3	5.6	15.6
25/06/2017	26/06/2017	0	10.7	6.4	15.5
26/06/2017	27/06/2017	0	9.8	5.6	16.4
27/06/2017	28/06/2017	0	9.7	5.5	15.8
28/06/2017	29/06/2017	0.4	11.1	7.6	16.8
29/06/2017	30/06/2017	0	7.8	2.8	16.3
30/06/2017	1/07/2017	0	9.8	6.1	15
1/07/2017	2/07/2017	42.6	14	10.2	15.2
2/07/2017	3/07/2017	18	11.5	7.4	15.6
3/07/2017	4/07/2017	0	10.4	6.2	16.2
4/07/2017	5/07/2017	0	10.2	4.5	16.3
5/07/2017	6/07/2017	3.6	10.8	7.5	15.2
6/07/2017	7/07/2017	13.2	10.7	10.1	11.3
7/07/2017	8/07/2017	0.4	10.1	6.5	13
8/07/2017	9/07/2017	16	11.2	8.8	14.8
9/07/2017	10/07/2017	0	11.6	9.9	14.4
10/07/2017	11/07/2017	0	10.7	4.7	16.4
11/07/2017	12/07/2017	1.6	8.8	3.7	15.7
12/07/2017	13/07/2017	0.2	7.1	1.2	13.8
13/07/2017	14/07/2017	1	7	4.5	9
14/07/2017	15/07/2017	6.6	8.9	4.9	11.7
15/07/2017	16/07/2017	0	7.2	0.2	14.7
16/07/2017	17/07/2017	0	9.2	4	16.6
17/07/2017	18/07/2017	0	12.2	8.1	15.5
18/07/2017	19/07/2017	0.2	12.6	9.5	17.1
19/07/2017	20/07/2017	1	12.9	9.3	17.4
20/07/2017	21/07/2017	51	14.2	11.8	16.3
21/07/2017	22/07/2017	10	11.7	7.6	14.9
22/07/2017	23/07/2017	0.2	10.3	5.5	14.5
23/07/2017	24/07/2017	0	10.6	6.5	15.6
24/07/2017	25/07/2017	0	10.5	6.2	15.9
25/07/2017	26/07/2017	0	9.9	2.8	17
26/07/2017	27/07/2017	0	9.8	4.7	16.8
27/07/2017	28/07/2017	11.4	11.9	8.8	16.4
28/07/2017	29/07/2017	0	8.2	1.8	13.6
29/07/2017	30/07/2017	0	5.3	-1.2	12.3
30/07/2017	31/07/2017	0	4.7	-0.2	12.9
31/07/2017	1/08/2017	0	7.4	2	13.4
1/08/2017	2/08/2017	0	11.2	9.4	13.8
2/08/2017	3/08/2017	9.4	11	9.3	13.2
3/08/2017	4/08/2017	0	8.3	2.9	15.8
4/08/2017	5/08/2017	0	8	4.4	14.3
5/08/2017	6/08/2017	0	8.3	3.3	16.2

Start date	Stop date	Rain total	Temperature dry bulb mean	Temperature DRY BULB MIN	Temperature dry bulb max
6/08/2017	7/08/2017	17.4	13	6.9	14.4
7/08/2017	8/08/2017	0	13.5	9.9	20
8/08/2017	9/08/2017	12.4	14.6	12.7	15.7
9/08/2017	10/08/2017	27.8	13.4	8.3	15.6
10/08/2017	11/08/2017	1.2	10.7	5.9	17.1
11/08/2017	12/08/2017	0	13.1	11	16.6
12/08/2017	13/08/2017	0	14.1	9.8	19.2
13/08/2017	14/08/2017	0	13.4	7.9	18.7
14/08/2017	15/08/2017	1	10.5	4.6	17.2
15/08/2017	16/08/2017	0.4	10.2	5.8	15.8
16/08/2017	17/08/2017	0.6	11.4	7.5	15.9
17/08/2017	18/08/2017	0.4	12.7	6.9	17.5
18/08/2017	19/08/2017	19.2	13.6	11.1	18.4
19/08/2017	20/08/2017	4.4	10.5	6.9	15.3
20/08/2017	21/08/2017	0.6	11.2	8.3	14.9
21/08/2017	22/08/2017	4.6	10.6	6.2	16.8
22/08/2017	23/08/2017	0	8.7	3.2	15.5
23/08/2017	24/08/2017	0	8.6	2.6	16.3
24/08/2017	25/08/2017	0	10	5	16.7
25/08/2017	26/08/2017	2.8	11.9	8.3	17
26/08/2017	27/08/2017	5.4	11.8	6.3	16.3
27/08/2017	28/08/2017	9.6	14.8	13.6	16.5
28/08/2017	29/08/2017	72	14.3	11.4	15.7
29/08/2017	30/08/2017	0	10.4	-0.3	18.9
30/08/2017	31/08/2017	23.8	12.1	10.3	15.1
31/08/2017	1/09/2017	1.6	11.6	7.9	17.2
1/09/2017	2/09/2017	41.4	12.1	8.9	16.4
2/09/2017	3/09/2017	17.8	10.2	9.6	12.6
3/09/2017	4/09/2017	0	11	7.5	14.8
4/09/2017	5/09/2017	0	11.2	4.4	17.4
5/09/2017	6/09/2017	0.6	13.6	11.7	16.3
6/09/2017	7/09/2017	13.6	13.2	11.6	15
7/09/2017	8/09/2017	5.4	11.3	7.7	17
8/09/2017	9/09/2017	4.8	11.6	8.6	15.2
9/09/2017	10/09/2017	12.4	8.4	3.2	13.2
10/09/2017	11/09/2017	3.2	6.8	3.1	11.8
11/09/2017	12/09/2017	1.4	7.6	4	13.1
12/09/2017	13/09/2017	0	11	9.2	14.5

Appendix 6. Te Puke Research Centre weather during the grafting trial

Mean, maximum and minimum daily temperatures (°C) and daily rainfall (mm) at Te Puke Research Centre, Bay of Plenty, during the grafting trial from 21 June to 5 July 2017.

Start date	Stop date	Rain total	Temperature dry bulb mean	Temperature dry bulb min	Temperature dry bulb max
20/06/2017	21/06/2017	0	12	7.6	18.1
21/06/2017	22/06/2017	10.8	13.4	11.8	15
22/06/2017	23/06/2017	49.6	14.4	13.4	16.4
23/06/2017	24/06/2017	25.2	15.7	13.3	17.4
24/06/2017	25/06/2017	0	11.3	5.6	15.6
25/06/2017	26/06/2017	0	10.7	6.4	15.5
26/06/2017	27/06/2017	0	9.8	5.6	16.4
27/06/2017	28/06/2017	0	9.7	5.5	15.8
28/06/2017	29/06/2017	0.4	11.1	7.6	16.8
29/06/2017	30/06/2017	0	7.8	2.8	16.3
30/06/2017	1/07/2017	0	9.8	6.1	15
1/07/2017	2/07/2017	42.6	14	10.2	15.2
2/07/2017	3/07/2017	18	11.5	7.4	15.6
3/07/2017	4/07/2017	0	10.4	6.2	16.2
4/07/2017	5/07/2017	0	10.2	4.5	16.3
5/07/2017	6/07/2017	3.6	10.8	7.5	15.2

Appendix 7. BBCH scores for Gold3 potted plants

Table 7.1. Data for callus formed at the wound site, cane dieback from wound site, dieback stop at a node or shoot from kiwifruit plants *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) treated with different wound protectant treatments at Te Puke Research Station.

Treatment	Rep.	Callus*	Dieback (mm)	Dieback stop**
1. Copper sulphate pentahydrate	1	N	105	**1**
	2	N	140	2
	3	N	90	2
	4	N	90	1
	5	N	270	1.5
2. Nordox™ 75WG	1	N	40	1
	2	N	120	2
	3	N	30	1
	4	N	40	1
	5	N	40	1
3. Greenseal™ Ultra	1	N	30	1
	2	N	20	1
	3	N	50	1
	4	N	90	1
	5	N	45	1
4. Topsin® M-4A	1	N	90	1
	2	N	20	1
	3	N	90	1
	4	N	40	1
	5	N	30	1
5. Damar® biological	1	N	100	2
	2	N	15	1
	3	N	45	1
	4	N	15	1
	5	N	50	1.5
6. New InocBloc™ paste	1	N	35	1
	2	N	45	1
	3	N	30	1
	4	N	40	1
	5	N	20	1
7. InocBloc™ paste	1	N	100	1
	2	N	15	1
	3	N	45	1
	4	N	15	3
	5	N	50	1
8. Inoculated wounded control	1	N	35	1
	2	N	20	1
	3	N	140	1
	4	N	150	2
	5	N	50	1
9. Uninoculated wounded control	1	N	180	2
	2	N	15	1
	3	N	30	1
	4	N	30	1
	5	N	40	1

*= Yes (Y) or No (N) if any callus has formed at the wound site
**= **node/shoot or area which die back from wound site ceases, typically 1st node/shoot below wound site.

Table 7.2. Values for the number of nodes in shoot 1 and their development according to the BBCH stage described by Salinero et al. (2009) at the end of the trial (20 October 2017) from kiwifruit plants *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) treated with different wound protectant treatments at Te Puke Research Station.

Treatment	Rep	Shoot1 Dist	Shoot1 Nodes	Stage						
				00	**03**	**11**	**12-18**	**51**	**53**	**Dead**
1. Copper sulphate	1	10	1							1
pentahydrate	2	30	1							1
	3	90	1				1	1		
	4	90	4		3		1			
	5	40	1							1
2. Nordox™ 75WG	1	40	3		1		2		1	
	2	40	1						2	
	3	30	5		3		2			
	4	40	3		2		1			
	5	40	2				2			
3. Greenseal™ Ultra	1	30	4		3		1			
	2	20	1		1					
	3	50	3		2		1			
	4	90	8		4		4			
	5	45	1				1	1		
4. Topsin® M-4A	1	90	4		3		1		1	
	2	20	4		3	1				
	3	90	6		4		2			
	4	40	5		3		2		1	
	5	30	3				3			
5. Damar® biological	1	5	1							1
	2	15	5		3		2			
	3	45	5		3		2		1	
	4	15	5		3		2			
	5	25	1				1		1	
6. New InocBloc™ paste	1	35	4		3		1			
	2	45	3		2		1		1	
	3	30	5		3		3			
	4	40	5		4		1		1	
	5	20	7		3		2			
7. InocBloc™ paste	1	50	4			1	3			
	2	45	5		2		3			
	3	100	7		4		3			
	4	80	5							1
	5	60	4		2		2	1		
8. Inoculated control	1	35	7		4		3			
	2	20	2		1		1			
	3	140	1				1	1		
	4	60	1							1
	5	50	5		4		1			
9. Uninoculated control	1	80	1							1
	2	15	4	1	2		1			
	3	30	7	4			3		1	
	4	30	5	4			1	1		
	5	40	4	1			3		1	

Table 7.3. Values for the number of nodes in shoot 2 and their development according to the BBCH stage described by Salinero et al. (2009) at the end of the trial (20 October 2017) from kiwifruit plants *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) treated with different wound protectant treatments at Te Puke Research Station.

Treatment	Rep	Shoot2 Dist	Shoot2 Nodes	BBCH Stage						
				00	03	11	12-18	51	53	Dead
1. Copper sulphate	1	105	1				1			
pentahydrate	2	140	9		5		4			
	3	200	1		1					
	4	160	4		2		2			
	5	130	1							1
2. Nordox™ 75WG	1	100	3		2		1			
	2	120	5		3		2			
	3	120	1				1		1	
	4	130	1							1
	5	130	5		4		1		1	
3. Greenseal™ Ultra	1	190	5		4		1			
	2	95	1				1		1	
	3	100	1							1
	4	250	1				1	1		
	5	75	1		1					
4. Topsin® M-4A	1	210	1				1		1	
	2	100	5		4		1		1	
	3	290	1				1			
	4	170	4		2	1	1			
	5	105	3		2		1			
5. Damar® biological	1	100	5		4		1		1	
	2	70	1				1	1		
	3	180	1							1
	4	60	1							1
	5	85	1		1					
6. New InocBloc™ paste	1	100	7		4		3		1	
	2	130	3		2		1		1	
	3	170	1				1			
	4	110	5		2		3	1		
	5	40	1				1			
7. InocBloc™ paste	1	180	1				1		1	
	2	160	1				1	1		
	3	300	1				1			
	4	130	5							1
	5	120	1							1
8. Inoculated control	1	70	4		2	1	1			
	2	110	2		1		1			
	3	260	1				1			
	4	150	6		4		2	1		
	5	140	4		2		2			
9. Uninoculated control	1	180	2				2			
	2	70	5		4		1			
	3	110	1				1			
	4	110	2		1		1			
	5	100	4							

Appendix 8. BBCH scores for 'Hayward' potted plants

Table 8.1. Data for callus formed at the wound site, cane dieback from wound site, dieback stop at a node or shoot from *Actinidia chinensis* var. *deliciosa* 'Hayward' plants treated with different wound protectant treatments at Te Puke Research Station. Replicates were not sampled for PCR.

Treatment	Rep.	Callus*	Dieback (mm)	Dieback stop**
1. Copper sulphate pentahydrate	3	N	100	2
	4	N	4	1
	6	N	70	1
	8	N	65	1
	10	N	130	5
2. Nordox™ 75WG	3	N	2	1
	4	N	2	1
	6	N	95	2
	8	N	30	1
	10	N	4	1
3. Greenseal™ Ultra	3	N	55	1
	4	N	1	1
	6	N	0	1
	8	N	25	1
	10	N	4	1
4. Topsin® M-4A	3	N	80	2
	4	N	5	1
	6	N	10	1
	8	N	10	1
	10	N	7	1
5. Damar® biological	3	N	18	1
	4	N	300	3
	6	N	3	1
	8	N	0	1
	10	N	4	1
6. New InocBloc™ paste	3	N	4	1
	4	N	5	1
	6	N	3	1
	8	N	43	1
	10	N	10	1
7. InocBloc™ paste	3	N	5	1
	4	N	2	1
	6	N	60	1
	8	N	12	1
	10	N	5	1
8. Inoculated wounded control	3	N	2	1
	4	N	3	1
	6	N	103	2
	8	N	7	1
	10	N	20	1
9. Uninoculated wounded control	3	N	0	1
	4	N	2	1
	6	N	2	1
	8	N	2	1
	10	N	40	2

*= Yes (Y) or No (N) if any callus has formed at the wound site
**= **node/shoot or area which die back from wound site ceases, typically 1st node/shoot below wound site.

Table 8.2. Values for the number of nodes in shoot 1 and their development according to the BBCH stage described by Salinero et al. (2009) at the end of the trial (19 December 2017) from *Actinidia chinensis* var. *deliciosa* 'Hayward' plants treated with different wound protectant treatments at Te Puke Research Station. Replicates were not sampled for PCR.

Treatment	Rep	Shoot1 Dist	Shoot1 Nodes	Stage								
				00	**01**	**03**	**07**	**09**	**11**	**12-18**	**19**	**Dead**
1. Copper sulphate	3	180	6							6		
pentahydrate	4	140	1		1							
	6	25	1									1
	8	90	2								2	
	10	90	1			1						
2. Nordox™ 75WG	3	25	2						2			
	4	5	1		1							
	6	20	1									1
	8	30	1								1	
	10	17	1			1						
3. Greenseal™ Ultra	3	0	0									
	4	0	0									
	6	1	3								3	
	8	19	1								1	
	10	45	1					1				
4. Topsin® M-4A	3	0	0									
	4	150	1		1							
	6	75	4								4	
	8	110	3								3	
	10	10	1			1						
5. Damar® biological	3	80	1									1
	4	100	1		1							
	6	45	4							4		
	8	1	1			1						
	10	40	4							4		
6. New InocBloc™ paste	3	0	0									
	4	20	1					1				
	6	8	2								2	
	8	45	7								7	
	10	33	1			1						
7. InocBloc™ paste	3	25	1		1							
	4	0	0									
	6	16	5							5		
	8	30	2								2	
	10	10	4							4		
8. Inoculated control	3	0	0									
	4	0	0									
	6	170	1								1	
	8	50	5	1							3	1
	10	42	1			1						
9. Uninoculated control	3	80	1		1							
	4	30	1		1							
	6	60	9								9	
	8	10	1								1	
	10	40	2							2		

Table 8.3. Values for the number of nodes in shoot 2 and their development according to the BBCH stage described by Salinero et al. (2009) at the end of the trial (19 December 2017) from *Actinidia chinensis* var. *deliciosa* 'Hayward' plants treated with different wound protectant treatments at Te Puke Research Station. Replicates were not sampled for PCR.

Treatment	Rep	Shoot2 Dist	Shoot2 Nodes	Stage							
				00	**01**	**03**	**09**	**11**	**12-18**	**19**	**Dead**
1. Copper sulphate	3	0	0								
pentahydrate	4	230	1					1			
	6	130	2		1					1	
	8	155	1							1	
	10	100	1			1					
2. Nordox™ 75WG	3	305	1		1						
	4	0	0								
	6	75	1			1					
	8	110	2							2	
	10	35	3	3							
3. Greenseal™ Ultra	3	0	0								
	4	0	0								
	6	45	3							3	
	8	125	1							1	
	10	75	1			1					
4. Topsin® M-4A	3	0	0								
	4	235	1		1						
	6	145	4			1				3	
	8	160	3							3	
	10	27	1				1				
5. Damar® biological	3	150	3						3		
	4	170	1		1						
	6	120	1						1		
	8	20	1			1					
	10	65	4						4		
6. New InocBloc™ paste	3	0	0								
	4	100	1					1			
	6	100	2							2	
	8	0	0								
	10	55	1			1					
7. InocBloc™ paste	3	80	1		1						
	4	0	0								
	6	185	1						1		
	8	75	3							3	
	10	45	1		1						
8. Inoculated control	3	0	0								
	4	0	0								
	6	220	1							1	
	8	135	4							4	
	10	60	1			1					
9. Uninoculated control	3	80	1		1						
	4	30	1		1						
	6	60	1						1		
	8	10	3							3	
	10	40	1							1	

Table 8.4. Data for callus formed at the wound site, cane dieback from wound site, dieback stop at a node or shoot from *Actinidia chinensis* var. *deliciosa* 'Hayward' plants treated with different wound protectant treatments at Te Puke Research Station and sampled for PCR testing 6 weeks after treatments were applied..

Treatment	Rep.	Callus*	Dieback (mm)	Dieback stop**
1. Copper sulphate pentahydrate	1	N	600	3
	2	N	940	7
	5	N	1	1
	7	N	2	1
	9	N	4	1
2. Nordox™ 75WG	1	N	290	4
	2	N	750	9
	5	N	93	4
	7	N	405	6
	9	N	0	1
3. Greenseal™ Ultra	1	N	470	4
	2	N	820	10
	5	N	0	1
	7	N	1	1
	9	N	2	1
4. Topsin® M-4A	1	N	1300	7
	2	N	5	1
	5	N	1	1
	7	N	3	1
	9	N	12	1
5. Damar® biological	1	N	850	8
	2	N	820	8
	5	N	2	1
	7	N	1	1
	9	N	0	1
6. New InocBloc™ paste	1	N	690	5
	2	N	730	5
	5	N	1	1
	7	N	0	1
	9	N	1	1
7. InocBloc™ paste	1	N	860	6
	2	N	960	8
	5	N	1	1
	7	N	0	1
	9	N	0	1
8. Inoculated wounded control	1	N	840	8
	2	N	695	6
	5	N	1	1
	7	N	1	1
	9	N	110	4
9. Uninoculated wounded control	1	N	720	14
	2	Y	3	1
	5	N	1	1
	7	N	0	0
	9	N	0	1

*= Yes (Y) or No (N) if any callus has formed at the wound site
**= **node/shoot or area which die back from wound site ceases, typically 1st node/shoot below wound site.

Table 8.5. Values for the number of nodes in shoot 1 and their development according to the BBCH stage described by Salinero et al. (2009) at the end of the trial (19 December 2017) from *Actinidia chinensis* var. *deliciosa* 'Hayward' plants treated with different wound protectant treatments at Te Puke Research Station and sampled for PCR testing 6 weeks after treatments were applied..

Treatment	Rep	Shoot1 Dist	Shoot1 Nodes	Stage								
				00	01	03	07	09	11	12-18	19	Dead
1. Copper sulphate	1	0	5									5
pentahydrate	2	0	0							0		
	5	70	5							5		
	7	15	4								4	
	9	23	1						1			
2. Nordox™ 75WG	1	340	3									
	2	0	0									
	5	180	1						1			
	7	460	4			1						
	9	10	12	12								
3. Greenseal™ Ultra	1	500	6								6	
	2	0	0									
	5	0	0									
	7	150	1								1	
	9	32	3							3		
4. Topsin® M-4A	1	0	0									
	2	640	4							4		
	5	5	1	1								
	7	55	2								2	
	9	18	1			1						
5. Damar® biological	1	0	8									8
	2	0	0									
	5	3	1	1								
	7	45	2								2	
	9	12	1			1						
6. New InocBloc™ paste	1	660	5									5
	2	0	0									
	5	70	7							7		
	7	50	1			1						
	9	20	2			1					1	
7. InocBloc™ paste	1	0	6									6
	2	0	0									
	5	6	1					1				
	7	45	6				1				5	
	9	15	13	10			1				2	
8. Inoculated control	1	0	8									8
	2	0	0									
	5	45	7							7		
	7	22	3								3	
	9	150	5							5		
9. Uninoculated control	1	0	0									
	2	280	1		1							
	5	50	1			1						
	7	50	2								2	
	9	20	6							2	4	

Table 8.6. Values for the number of nodes in shoot 2 and their development according to the BBCH stage described by Salinero et al. (2009) at the end of the trial (19 December 2017) from *Actinidia chinensis* var. *deliciosa* 'Hayward' plants treated with different wound protectant treatments at Te Puke Research Station and sampled for PCR testing 6 weeks after treatments were applied..

Treatment	Rep	Shoot2 Dist	Shoot2 Nodes	Stage							
				00	01	03	09	11	12-18	19	Dead
1. Copper sulphate	1	-									
pentahydrate	2	0									
	5	160	3							3	
	7	140	1							1	
	9	70	3					3			
2. Nordox™ 75WG	1	900	2							2	
	2	0	0								
	5	182	1				1				
	7	40	1			1					
	9	25	5				1			4	
3. Greenseal™ Ultra	1	0									
	2	0									
	5	0	0								
	7	250	6								6
	9	70	1					1			
4. Topsin® M-4A	1	0									
	2	250						5	4		
	5	55	1								1
	7	150			1						
	9	42									
5. Damar® biological	1	-									
	2	0									
	5	25	4			2			2		
	7	100	1							1	
	9	64	5			2				3	
6. New InocBloc™ paste	1	700	9						9		
	2	-									
	5	0	0								
	7	150	2							2	
	9	60	1			1					
7. InocBloc™ paste	1	-									
	2	0									
	5	125	1				1				
	7	-									
	9	0									
8. Inoculated control	1	0									
	2	0									
	5	60				1		4			
	7	0							5		
	9	100									
9. Uninoculated control	1	0									
	2	-									
	5	25		1							
	7	110					3				
	9	0									

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