Risk of spreading Psa-V via wound protectants or application tools

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Aim
To determine the risk of spreading Psa-V between kiwifruit vines via wound protectants or application tools used during grafting and pruning.

Method

Lab testing
At the end of 2012, the wound protectants outlined in table 1 were tested to see if Psa-V was able to survive in them. A sub-sample of each product was sent to Plant and Food Research, where they were inoculated with Psa-V. Specifically, a suspension of Psa-V containing a known concentration of bacteria was mixed with an equal volume of each product (neat) with the resulting Psa-V populations determined by 1/10th dilutions plated on King's B medium.

In experiment 1, the products were inoculated with a Psa-V concentration of 4.3×10^{9} cfu ml^{-1} with the amount of Psa-V quantified 17.5 hours and 24 hours later. In experiment 2, those products that demonstrated no survival of Psa-V after 17.5 hours were retested. Products were inoculated with Psa-V at 1.4×10^{10} cfu ml^{-1} with the amount of Psa-V quantified 15, 30, 60 and 120 minutes after inoculation.

Table 1 Wound protectants assessed for supporting Psa-V survival in the laboratory.

Product	**Active Ingredients**
Smiths Wax	wax
Gripset Betta	bitumen rubber
GreenSeal Ultra	tebuconazole + octhilinone
Garrison NF	cyproconazole + iodocarb
BacSeal Super	tebuconazole

Field testing
The wound protectants outlined in table 2 were applied in August 2012 during grafting of a Gold3 orchard in Te Puke. Each product was decanted into 5 sub-sample pots (reps) and for each pot a clean application tool was used. The wax was applied with a kitchen butter knife; all other products were applied with a small paint brush (Figure 1).

When applying, the tools were dipped into their respective product pots and swiped at least two times over the cut area of each grafted stump. For each pot and tool, four vines were treated, resulting in 20 stumps being treated for each product i.e. 5 reps x 4 stumps/rep. After use, the tools were bagged and pots sealed and transported in person by the sampler to Plant and Food Research in Ruakura to determine whether Psa-V could be isolated from them. 1 sample from each treatment was analysed once they arrived (late afternoon) with the other 4 samples analysed the next morning.

Table 2 Wound protectant treatments tested in the field.

	Treatment	No. of pots (replicates)	Application tool	No. of application tools (replicates)
1	BacSeal Super	5	Brush	5
2	BacSeal Super + Nordox (4g/L)	5	Brush	5
3	GreenSeal Ultra	5	Brush	5
4	Garison NF	5	Brush	5
5	Smith Wax	5	Kitchen knife	5
6	KeyStrepto hand applied spray followed by Smith Wax*	5	Kitchen knife	1
7	Gripset Betta	5	Brush	5

* The pots analysed were of the wax which would have received some KeyStrepto as a result of the application.

Figure 1. Sampling equipment used on orchard trial.

Results

Lab testing

In experiment 1, Psa-V could be isolated from the Smiths Wax, Gripset Betta and Garrison NF 17.5 hours after inoculation; Psa-V could also be isolated after 24 hours from the Gripset Betta and Garrison NF (table 3). Psa-V could not be isolated from GreenSeal Ultra or BacSeal Super at either time. In experiment 2, these two products were re-tested and isolations performed at shorter intervals. The shortest interval assessed was 15 minutes after inoculation and at this time no Psa-V could be isolated from GreenSeal Ultra (table 4). Psa-V was isolated from BacSeal Super 15 minutes after inoculation, but not at 30 minutes after inoculation.

Table 3 Lab experiment 1: amounts of Psa-V found in wound protectants following inoculation with a concentration of 4.3×10^9 cfu ml^{-1}.

Product	Bacterial count 17.5 hrs after inoculation (cfu ml^{-1})	Bacterial count 24 hrs after inoculation (cfu ml^{-1})
Smiths Wax	4.6×10^9	Not determined
Gripset Betta	5.6×10^6	6.0×10^6
GreenSeal Ultra	0	0
Garrison NF	1.8×10^9	1.3×10^9
BacSeal Super	0	0
Nil Treatment	1.7×10^9	1.5×10^9

Table 4 Lab experiment 2: amounts of Psa-V found in wound protectants that previously demonstrated no survival 17.5 hours after inoculation.

Product	15 minutes after inoculation (cfu ml^{-1})	30 minutes after inoculation (cfu ml^{-1})	60 minutes after inoculation (cfu ml^{-1})	120 minute safter inoculation (cfu ml^{-1})
Nil Treatment	7.7×10^9	7.7×10^9	7.0×10^9	6.3×10^9
GreenSeal Ultra	0	0	0	0
BacSeal Super	8.0×10^7	0	0	0

Field testing

Psa-V was detected in at least one of the 5 pots for the following treatments: BacSeal Super + Nordox, Smiths Wax and KeyStrepto + Smiths Wax treatment (Table 5). Psa-V was also detected on at least one of the tools used to apply the Bacseal Super, Garrison NF, Smith Wax and Gripset Betta. Of the 10 samples in which Psa-V was detected, 8 were plated the morning after they were received.

Table 5 Field trial results. Number of pots or application tools (out of 5) in which Psa-V was detected*.

	Treatment	Product in pot	Application tool
1	BacSeal Super	0	1#
2	BacSeal Super + Nordox	1	0
3	GreenSeal Ultra	0	0
4	Garrison NF	0	1#
5	Smith Wax	2	1
6	KeyStrepto hand applied spray followed by Smith Wax+	1	0
7	Gripset Betta	0	3

* 1 sample from each treatment was analysed the day of receipt with the other 4 analysed the next morning.

+ The pots analysed were of the wax which would have received some KeyStrepto as a result of the application.

These samples were plated the day of receipt. All other samples were plated the morning after receipt.

Conclusions

The results of this trial indicate there may be a risk of spreading Psa-V from vine to vine via some wound protectants and application tools. Minimise this risk by using a wound protectant containing a bactericide. In this preliminary study, GreenSeal Ultra was the most effective product tested for preventing Psa survival in the lab and field study.

Sampling and replication was limited in this study. Further replicated studies are therefore recommended to confirm the findings here.