

## ***Invitro* study of Psa survival in wound protectants**

S. Dowlut, T. Karnik, F. Brown, M. Judd

**VLS Project No E2013-10.**

**Dec 2013**

## Table of Contents

<b>EXECUTIVE SUMMARY</b> .....	<b>3</b>
<b>AIM</b> .....	<b>4</b>
<b>BACKGROUND</b> .....	<b>4</b>
<b>METHODOLOGY</b> .....	<b>4</b>
MINIMUM INHIBITORY CONCENTRATION (MIC) IN 0.85 % NORMAL SALINE.....	4
DETERMINATION OF PSA-V SURVIVAL AFTER INOCULATION OF PRODUCT (KILL RATE V/S TIME).....	5
QUANTIFICATION AND REPEATABILITY.....	5
<b>RESULTS</b> .....	<b>5</b>
MINIMUM INHIBITORY CONCENTRATION (MIC) IN 0.85 % NORMAL SALINE.....	5
DETERMINATION OF PSA-V SURVIVAL AFTER INOCULATION OF PRODUCT.....	6
QUANTIFICATION AND REPEATABILITY .....	6
<b>CONCLUSIONS</b> .....	<b>7</b>

## Executive summary

This study examined the efficacy of a number of wound protectants alone and in combination with other bactericides:

No	Products	Active ingredients
1	Bacseal Super	Tebuconazole
2	Garrison NF	Cyproconazole +Iodocarb
3	Greenseal Ultra	Tebuconazole + octhilinone
4	Bacseal Super + Nordox	Tebuconazole + Nordox
5	Bacseal Super + Streptomycin	Tebuconazole + Streptomycin
6	Smith Grafting wax + Nordox	Nordox
7	Bituproofs + Nordox	Bitumen rubber + nordox

Minimum inhibitory concentrations were determined in saline and showed Bacseal Super to be completely effective at a concentration of 20% while Garrison NF and Green Seal were effective at concentrations down to 5%. Surprisingly none of the *combinations* of Bacseal, Smith Grafting wax or Bituproofs with either Nordox or streptomycin showed complete kill.

Since wound protectants are usually applied *neat* the subsequent *duration of survival* tests were undertaken on 50% and 70% solutions of the 3 successful products. These tests showed Green Seal Ultra was completely effective after 45mins while Garrison NF and Bacseal Super were completely effective after 2 hours. All tests were undertaken using high concentrations of Psa ( $10^8$ - $10^9$  cfu/mL).

## Aim

The purpose of this trial was to carry out an *invitro* study of Psa survival in wound protectants.

## Background

Wound protectants are used to protect wounds following pruning and grafting. Kiwifruit vines are pruned to remove excess wood in summer and to structure the vine in winter. Pruning wound dressings generally attempt to seal the wound to prevent water loss, promote callousing and often contain a bactericide aimed at providing protection from bacterial infections. This report presents the evaluation of 7 wound protectants tested against Psa-V to determine their efficacy under laboratory conditions.

The products were tested to determine

- 1) its minimum inhibitory concentration (MIC)
- 2) Survival of Psa-V in protectant (Kill rate v/s time)
- 3) Quantification and repeatability

Table 1 below shows the list of products tested and the main active ingredients.

No	Products	Active ingredients
1	Bacseal Super	Tebuconazole
2	Garrison NF	Cyproconazole +Iodocarb
3	Greenseal Ultra	Tebuconazole + octhilinone
4	Bacseal Super + Nordox	Tebuconazole + Nordox
5	Bacseal Super + Streptomycin	Tebuconazole + Streptomycin
6	Smith Grafting wax + Nordox	Nordox
7	Bituproofs + Nordox	Bitumen rubber + nordox

Table 1: List of products and active ingredient

## Methodology

### 1. Minimum Inhibitory Concentration (MIC) in 0.85 % normal saline

This assay measures the activity of the product against a target bacterium. For the Minimum Inhibitory Concentration (MIC), the product was tested at ten different concentrations; 0.1%, 0.5%, 1%, 2%, 5%, 10%, 20%, 50%, 70% and 90% which were prepared in Psa suspension of known concentration- ranging between  $10^8$  and  $10^9$  cfu/mL. The tubes were incubated at  $25 \pm 2^\circ\text{C}$  for 48 hours. The minimum bactericidal concentration (MBC) of the product was determined by sub-culturing the contents of the tubes on Aitken media. The plates were then incubated at  $25 \pm 2^\circ\text{C}$  for 48 hours and

examined for signs of antimicrobial activity. Based on the results obtained in the dilution test, a working concentration of each product was determined.

### 2. Determination of Psa-V survival after inoculation of product (kill rate v/s time)

Once the minimum inhibitory concentration (MIC) was determined, the survival of Psa-V was conducted by inoculating each product with Psa followed by incubation at 25°C. The products were tested after incubation times of 15 minutes, 30 minutes, 1 hour, 3 hours, 5 hours and 8 hours following inoculation. At each time interval, the inoculated product was streaked to determine the kill rate versus time and a growth score was recorded. A set of controls were also conducted with only Psa solution and no product at each time interval.

### 3. Quantification and repeatability

Once, the window of concentration of product versus kill rate versus time was determined, the time was further narrowed. The Psa solution was quantified pre-use by serial dilution and plating on Aitken media and run in sets of 5 replicates. A positive control was set with only Psa-V solution. At the end of incubation, the 5 replicates were quantified by serial dilution and plating and plates were read 48 hours post incubation.

## Results

### 1. Minimum Inhibitory Concentration (MIC) in 0.85 % normal saline

The minimum inhibitory concentrations were measured using concentrations from 0.1 % to 90% of each product which covered the zone between growth and no-growth of Psa-V. Consequently a working concentration for each product was derived from the MIC and used for subsequent testing as per Table 2 below. Out of the 7 products tested, Bacseal super, Garisson NF and Green Seal Ultra showed efficacy against Psa-V. Surprisingly, when these same products were used in combination with Nordox™ or streptomycin they did **not** show any inhibitory action against Psa-V.

Product under test	Starting concentration	Quantification in cfu/mL	0.10%	0.50%	1%	5%	10%	20%	50%	90%	Working concentration
Bacseal Super	Neat	2 x 10 <sup>9</sup>	G	G	G	G	G	NG	NG	NG	>20 %
Garisson NF	Neat	2 x 10 <sup>9</sup>	G	G	G	NG	NG	NG	NG	NG	>5 %
Green Seal Ultra	Neat	2 x 10 <sup>9</sup>	G	G	G	NG	NG	NG	NG	NG	> 5%
Bacseal Super + Nordox	Neat + 75g/100 L (Nordox)	2 x 10 <sup>9</sup>	G	G	G	G	G	G	G	G	N/A
Bacseal Super+ Streptomycin	Neat +10 % streptomycin	3 x 10 <sup>8</sup>	G	G	G	G	G	G	G	G	N/A
Smith Grafting wax + Nordox	Neat + 75g/100 L (Nordox)	3 x 10 <sup>8</sup>	G	G	G	G	G	G	G	G	N/A
Bituproof plus + Nordox	Neat + 75g/100 L (Nordox)	3 x 10 <sup>8</sup>	G	G	G	G	G	G	G	G	N/A

Table 2: MIC in 0.85 % saline suspension

Key: **G – Growth** **NG- No growth**

## 2. Determination of Psa-V survival after inoculation of product

Since the wound protectants are generally used undiluted in the orchard, working concentrations of 50% and 70% were used instead of the minimum inhibitory concentration. Absolute kill was obtained after 1 hour incubation for Greenseal Ultra and 3 hours for Garisson NF and Bacseal Super. The same results were found at both working concentrations. The Psa-V solution used for this trial was at a high concentration of  $2 \times 10^9$  cfu/mL.

Product	Concentration of product	Psa solution in cfu/mL	Psa -V counts after inoculation					
			15 minutes	30 minutes	1 hour	3 hours	4.15 hours	7.45 hours
Garisson NF	50%	$2 \times 10^9$	G4	G4	G1	NG	NG	NG
Greenseal Ultra	50%	$2 \times 10^9$	G4	G4	NG	NG	NG	NG
Bacseal Super	50%	$2 \times 10^9$	G4	G4	G3	NG	NG	NG
Garisson NF	70%	$2 \times 10^9$	G4	G4	G1	NG	NG	NG
Greenseal ultra	70%	$2 \times 10^9$	G4	G4	NG	NG	NG	NG
Bacseal Super	70%	$2 \times 10^9$	G4	G4	G3	NG	NG	NG

Table 3: Determination of Psa-V survival after inoculation of product

Key: **G – Growth**      **NG- No growth**

## 3. Quantification and Repeatability

At 45 minutes, Psa-V could not be isolated from Greenseal Ultra. Subsequent repeated testing consistently gave the same result. Refer to Table 4a.

Greenseal Ultra	Control Psa solution in cfu/mL	45 mins after inoculation in cfu/mL	1hr after inoculation in cfu/mL
Replicate 1	$4 \times 10^8$	0	0
Replicate 2	$4 \times 10^8$	0	0
Replicate 3	$4 \times 10^8$	0	0
Replicate 4	$4 \times 10^8$	0	0
Replicate 5	$4 \times 10^8$	0	0

Table 4 a: Quantification and Repeatability Greenseal Ultra

At 2 hours, Psa-V could not be isolated from Bacseal Super and Garisson NF. Subsequent repeated testing supported these results. Refer to Table 4b and 4c.

Bacseal Super	Control Psa solution in cfu/mL	2 hours after inoculation in cfu/mL	3 hrs after inoculation in cfu/mL
Replicate 1	4 x 10 <sup>8</sup>	0	0
Replicate 2	4 x 10 <sup>8</sup>	0	0
Replicate 3	4 x 10 <sup>8</sup>	0	0
Replicate 4	4 x 10 <sup>8</sup>	0	0
Replicate 5	4 x 10 <sup>8</sup>	0	0

*Table 4 b: Quantification and Repeatability Bacseal Super*

Garrison NF	Control Psa solution in cfu/mL	2 hours after inoculation in cfu/mL	3 hrs after inoculation in cfu/mL
Replicate 1	4 x 10 <sup>8</sup>	0	0
Replicate 2	4 x 10 <sup>8</sup>	0	0
Replicate 3	4 x 10 <sup>8</sup>	0	0
Replicate 4	4 x 10 <sup>8</sup>	0	0
Replicate 5	4 x 10 <sup>8</sup>	0	0

*Table 4 c: Quantification and Repeatability Garrison NF*

## Conclusions

Out of the 7 wound protectant combinations tested, Greenseal Ultra, Bacseal super and Garrison NF showed absolute kill of Psa-V within few hours of application of the product.

### Product Testing Disclaimer

*Verified Lab Services is a trading division of Seeka Kiwifruit Industries Ltd.*

*Verified Lab Services and Seeka Kiwifruit Industries make no representation or guarantee as to the product tested and its effectiveness as a treatment for PSA. Results above may not be conclusive due to limitations of the testing method and limitations of the product. Limitations of the testing method include but are not limited to: inability to evaluate the efficacy of biological control agents, inability to demonstrate efficacy of products that cannot diffuse through agar or make broth cloudy, inability to demonstrate efficacy of plant elicitors*

*This publication has been prepared based on information available to Verified Lab Services and Seeka Kiwifruit Industries Limited at the time of publication. Any person using the information in this publication does so at their own risk. Verified Lab Services, Seeka Kiwifruit Industries Ltd, and their contractors, gives no warranties, representation or guarantees as to the accuracy and/or completeness of the information provided in this publication, including in relation to sample collection procedures, potential treatments and/or best treatment practice. Verified Lab Services, Seeka Kiwifruit Industries Ltd, and their contractors shall have no liability to any person for any loss arising from that person's reliance on the information and/or for any direct, indirect or consequential losses or damages arising out of or connected with the use of the enclosed information. No obligation is accepted or undertaken to update this or any other information or publicly release revisions to this document to reflect additional information, circumstances or changes in expectations which occur after the date of this document.*

**End of document.**

