

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

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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 ⁹	G	G	G	G	G	NG	NG	NG	>20 %
Garisson NF	Neat	2 x 10 ⁹	G	G	G	NG	NG	NG	NG	NG	>5 %
Green Seal Ultra	Neat	2 x 10 ⁹	G	G	G	NG	NG	NG	NG	NG	> 5%
Bacseal Super + Nordox	Neat + 75g/100 L (Nordox)	2 x 10 ⁹	G	G	G	G	G	G	G	G	N/A
Bacseal Super+ Streptomycin	Neat +10 % streptomycin	3 x 10 ⁸	G	G	G	G	G	G	G	G	N/A
Smith Grafting wax + Nordox	Neat + 75g/100 L (Nordox)	3 x 10 ⁸	G	G	G	G	G	G	G	G	N/A
Bituproof plus + Nordox	Neat + 75g/100 L (Nordox)	3 x 10 ⁸	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 2×10^9 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%	2×10^9	G4	G4	G1	NG	NG	NG
Greenseal Ultra	50%	2×10^9	G4	G4	NG	NG	NG	NG
Bacseal Super	50%	2×10^9	G4	G4	G3	NG	NG	NG
Garisson NF	70%	2×10^9	G4	G4	G1	NG	NG	NG
Greenseal ultra	70%	2×10^9	G4	G4	NG	NG	NG	NG
Bacseal Super	70%	2×10^9	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	4×10^8	0	0
Replicate 2	4×10^8	0	0
Replicate 3	4×10^8	0	0
Replicate 4	4×10^8	0	0
Replicate 5	4×10^8	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 ⁸	0	0
Replicate 2	4 x 10 ⁸	0	0
Replicate 3	4 x 10 ⁸	0	0
Replicate 4	4 x 10 ⁸	0	0
Replicate 5	4 x 10 ⁸	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 ⁸	0	0
Replicate 2	4 x 10 ⁸	0	0
Replicate 3	4 x 10 ⁸	0	0
Replicate 4	4 x 10 ⁸	0	0
Replicate 5	4 x 10 ⁸	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.

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