Invitro study of Psa survival in PruneTec (Wound protectant)

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Aim

The purpose of this trial was to carry out an invitro study of Psa survival in wound protectant (PruneTec).

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 contain a bactericide that aimed at providing protection from bacterial infections. This report presents the evaluation of PruneTec 20001948 wound protectant tested against Psa-V to determine 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	Specification	Active ingredients
1	PruneTec	20001948	Tebuconazole

Table 1: List of product and active ingredient

Methodology

1. **Agar susceptibility**

A standard petri dish containing Mueller-Hinton CLSI agar was used for product testing. A 12 mm core of agar was excised from the surface of the plate. 30µL aliquots of the product (neat) were introduced into the excised holes and allowed to diffuse for 10 minutes before Psa was applied. An inoculum of Psa was prepared in sterile distilled water to a concentration of 0.5 McFarland Standard. This was evenly spread over the entire surface of the plate using a swab. The inoculated plate was incubated at 25 ± 2 °C for 48 hours and examined for signs of antimicrobial activity.

2. **Minimum Inhibitory Concentration (MIC) in sterile distilled water**

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 10^7 and 10^8 cfu/mL. The tubes were incubated at 25 ± 2°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 and blood agar. The plates were then incubated at 25 ± 2°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.

3. 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 the 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 and 7 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.

4. 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. Agar Susceptibility

No	Products	Concentration	Zone of inhibition
1	PruneTec 20001948	Neat	Yes

Table 2: Agar susceptibility test

Fig 1: PruneTec 20001948 Agar susceptibility test

2. Minimum Inhibitory Concentration (MIC) in sterile distilled water

The minimum inhibitory concentration of the product tested from 0.1 % to 90% dilution is shown in Table 3. A range of concentrations where both growth and no-growth of Psa-V was obtained and a working concentration for the product, derived from the MIC was used for subsequent testing. PruneTec 20001948 showed efficacy against Psa-V at or greater than 5 %.

Product under test	Starting concentration	Quantification in cfu/mL	0.10%	0.50%	1%	5%	10%	20%	50%	90%	Working concentration
PruneTec 20001948	Neat	1 x 10^8	G	G	G	NG	NG	NG	NG	NG	≥ 5 %

Table 3: MIC in sterile distilled water
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, a working concentration of 50% and 70% were used instead of the minimum inhibitory concentration. Absolute kill was obtained within 1hour incubation. The Psa-V solution used for this trial was at a high concentration of 1 x 10^8 cfu/mL.

			Psa-V counts after inoculation				
Product	Concentration of product	Psa solution in cfu/mL	15 mins	30 mins	1 hour	3 hours	7 hours
PruneTec 1948	50%	1 x 10^8	G	G	NG	NG	NG
PruneTec 1948	70%	1 x 10^8	G	G	NG	NG	NG

Table 4: Determination of Psa-V survival after inoculation of product

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

3. Quantification and Repeatability

At 1 hour, Psa-V could not be isolated from PruneTec 20001948. Subsequent repeated testing indicated the same result. Refer to Table 5a.

PruneTec 20001948	Control Psa solution in cfu/mL	1hr after inoculation in cfu/mL
Replicate 1	5.2 x 10^7	0
Replicate 2	5.2 x 10^7	0
Replicate 3	5.2 x 10^7	0
Replicate 4	5.2 x 10^7	0
Replicate 5	5.2 x 10^7	0

Table 5a: Quantification and Repeatability PruneTec 20001948

Conclusions

PruneTec 20001948 showed absolute kill to Psa-V within an hour of incubation.

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