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Disinfectant efficacy testing

VLS Project No: E2012-04

Report prepared for Zespri by

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Executive Summary

This trial tested 12 products for their efficacy as disinfectants against Psa. The aim was to provide practical advice to the industry for use where tools or equipment needs to be free from live Psa. It looks at the efficacy associated with both spraying and dipping (shallow immersion) for times from 10 seconds through to 2 minutes on 4 surfaces common within the industry:

- wood
- mild steel
- plastic
- rubber

The range of times was chosen as "practical" or "useable*"* for situations which are either *in-line* or where an operator may have to wait for that duration.

Working concentrations were chosen on the basis of *in-vivo* tests with some consideration given to practicalities i.e. it is likely costs and safety would prevent most disinfectants being used neat or at very high concentrations.

In addition to these straight efficacy tests we have recorded the pH of each product at its working concentration and attempted to provide an indication of the sensitivity of each product to both pH changes and the presence of organic matter. In any fruit application, pH may be critical of itself and in many situations there may be an abundance of organic matter in the form of soil or plant debris.

The following table summarises key results listing the products, their working concentrations and the ***minimum*** time (that we used) required for kill efficacy for each material for both spray and dip applications. Two tested products showed no efficacy as disinfectants under our test conditions.

Summary				Sensitive to...		Spray efficacy				Dip efficacy			
Product tested	Conc^{n}	pH	Likely Residue	pH	OM	Wood	Plastic	Tyre	Metal	Wood	Plastic	Tyre	metal
Envirosan	1%	6.9	Yes	B	NS	10s	1 min	NE	10s	10s	10s	NE	10s
Trigene	1%	7.3	Yes	NS	NS	30s	NE	NE	10s	10s	NE	NE	10s
Citrox	1%	6.4	No	NS	NS	10s	10s	NE	2 min	10s	1min	NE	2min
Janola	1%	8.4	No	NS	S	10s	10s	10s	10s	10s	10s	10s	10s
Virkon	1%	4	Yes	NS	S	10s	10s	10s	10s	10s	30s	10s	10s
H_2O_2	3%	6.8	No	NS	NS	10s	NE	2min	10s	10s	2min	NE	10s
Teracep	1%	4.8	No	NS	S	10s	10s	10s	10s	10s	30s	3s	10s
Kiwilustre	1%	4.1	No	S	NS	10s	NE	NE	30s	10s	10s	10s	2min
Extinct pure	1%	4	No	NS	NS	10s	2min	NE	10s	10s	2min	NE	10s
Citric acid	3%	2.5	No	S	NS	10s	10s	30S	10s	10s	10s	10s	10s
Zoono	10%	7.03	Yes	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Analyte	10%	7.3	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Abbrevs: NE = Not Effective; NS = Not sensitive; S = Sensitive, B=sensitive to *basic* conditions.

Table 1. Summary of results.

Finally we should note that any investigation of this type, particularly when it includes new or novel assessment procedures, is likely to raise further questions or the desire to push some boundaries further. This is certainly the case with this work where the product list, concentrations, times, Psa threat concentrations and sensitivities to organic matter and pH could all be reasonably extended. Given the constraints on time for this project such questions must be left for further study.

Disinfectant efficacy testing

VLS Project No: E2012-04

1.0 Aim

The purpose of this trial is to determine the efficacy of products used for sanitisation of surfaces.

2.0 Background

Both the post-harvest sector and growers invested considerable resources into orchard sanitation last season in the hope of reducing the risk of *Pseudomonas syringae* pv *actinidiae* (Psa V) infection and slowing its spread. Nevertheless there remain on-going concerns as to the efficacy, utility and cost-effectiveness of these efforts particularly those associated with harvest activities. An assessment of the efficacy of sanitising agents against Psa has become increasingly important stepping into the second season of the spread of Psa in the kiwi-industry.

This experiment was conducted to determine the efficacy of disinfecting products for sanitising

- field tools: secateurs, loppers and handsaws
- bins: both wood and plastic
- rubber tyres: motorbikes, tractors, trailers and trucks.

Previous work has shown the efficacy of sanitising products differs depending on the surfaces to which they are applied. To represent each of these common surfaces, small discs were created from untreated undressed timber, plastic (as used in some bins), rubber (from tyres) and mild steel.

This report presents the evaluation of 12 *potential* disinfectants tested both in-vitro and in spray and dip applications on all 4 surfaces after each surface had been *spiked* with Psa V. While undertaken with time constraints this project includes a number of different testing approaches. Initial tests were undertaken in solution (both broth and saline) to determine appropriate concentrations for efficacy. These procedures were modified according to the amount of available information. If earlier work had demonstrated efficacy around a given concentration range we were able to focus our attention on a tight range of concentrations whereas if little was known our initial assessment was spread over a wider concentration range.

We then looked at "kill versus time" efficacy using the spiked discs. Times were restricted to relatively short *practical* durations – there seemed little point in testing over times longer than a few minutes since any in-line procedure, or one where an operator was required to wait for that duration of the process, seemed unlikely to be effectively implemented.

The sensitivity of each product to acidity (pH) was then assessed by slowly adding a simple inorganic (dissociated) acid while monitoring the pH in order to adjust the solution acidity to several predetermined levels. The bactericidal activity was tested at each pH level.

Finally, we attempted to assess whether the efficacy of the disinfectant was affected by the presence of organic matter. A fixed amount of tannic acid was added to a quantum of each product and its efficacy again assessed. In reality there may be no upper limit to organic matter contamination (e.g. soil on bins). Given the required duration and the scale of this project the aim of this test was to indicate whether each product's efficacy is likely to be affected by the presence of organic matter to

act as a warning if the material is going to be used in a situation where organic matter contamination is likely.

Microbiological tests as used in this project often realise *outliers*. Results fall into this category if they do not seem to align with other tests or prior expectations. These are highlighted and acknowledged within the report but they have not been unravelled. Finally, the nature of this type of investigation, particularly when novel assessment techniques have been introduced, is that the results may raise further questions either of interest or of explanation. We have not attempted to pursue these questions; they must remain the subject of further work.

3.0 Materials and Equipment

ICMP culture strain no 18800 and 18708 (Psa V strain), Psa V media, Kings B media, Kings B broth, Trptic soy broth (TSB), spray bottles, sterile distilled water, spreaders, sterile swabs, disposable tips, stop watch, 0.85 % saline, PCR, serological pipettes (10 /25 ml), transfer pipettes, sterile 15 ml tubes, 0.1 M HCl, tannic acid, phosphate buffers.

3.1 Plating media

Since last year, Verified Lab Services (VLS) has been using a media developed by J Aitken, member of TaskForce Green which is selective to Psa V. This considerably assists the identification of Psa V through morphological characteristics since many other bacteria and fungi do not grow on it. Throughout this project we used both this Psa selective media alongside Kings B (which is less selective among the *Pseudomonas sp*) for all plating studies.

3.2 Products

The products tested were either suggested by Zespri/KVH or by VLS. Of course they represent only a small fraction of available possibilities however all parties were canvassed for their suggestions prior to finalising this list. Refer to Table 1 below.

3.3 Test Surfaces

Twelve mm diameter discs were prepared of the following substances:

- Untreated, undressed wood (pine)
- Plastic
- Mild steel
- Rubber (from tyres

Product	Active Ingredients	Comment
Envirosan	Glutaral, didecyldimethylammonium chloride, propan-2 –o, methanol	[1]
Trigene	Polymeric (Hexamethylene) Biguanide hydrochloride alkyldimethyl benzyl dimethy ammonium chloride, Dodecylamine sulphamat	[1]
Citrox PWT	Citrus pulp extract, water (demineralised), Citric acid, Glycerin	No likely residuals – plant extract
Janola	Sodium hypochlorite, sodium hydroxide	No likely residual
Virkon	Potassium peroxomonosulphate, sodium dodecylbenzene sulphonate, sulfamic acid	Possibly
Hydrogen peroxide	Hydrogen peroxide 20-40 %, water	No likely residual
Teracep	Paracetic acid, hydrogen peroxide, acetic acid	No likely residual
Kiwilustre	Phosphate buffered lactic acid	No likely residual[2]
Citric acid	Citric acid 100 %	No likely residual
Extinct pure	Chlorine dioxide	No likely residual
Analyte	Hypochlorous acid (HClO) and Hypochlorite ion (OCl-)	No likely residual
Zoono	3-Trimethoxy silyl propyl dimethyl octadecyl Ammonium Chloride	[1]

Notes:

1. Contains quarternary ammonium compounds that may cause residues unless precautions are taken e.g. adequate drying times.

2. Kiwilustre may leave a residue however this is acceptable to the industry and to consumers. It is commonly applied last thing before harvest to *clean-up* fruit.

Table 1. List of products tested and their active ingredients.

Comments regarding the likelihood of residues are made from observations of the active ingredients only. They are ***not*** based on observation or measurement so should be treated with caution and not relied on for critical use without verification.

4.0 Methodology

4.1 Preparation of Psa-suspension in 0.85 % saline (high concentration)

1 ml of ICMP culture 18800 Psa V was inoculated in 99 ml TSB broth and allowed to incubate at 25°C ± 2°C. After 48 hrs, the TSB broth showed growth. The level of growth was quantified using a serial dilution method using Kings B plates and 0.85% saline. After 2 days growth in TSB, 100 µl was inoculated onto Psa V media and allowed to grow for48 hours. After 48 hours, the colonies were re-suspended in 0.85% saline solution. About 1 litre of this inoculum was made for the purpose of this experiment.

4.2 Minimum Inhibitory Concentration (MIC) in broth

This assay measures the activity of a chemical agent against Psa growing in a nutritional broth i.e. a complex, relatively poorly defined, bacteria beneficial, environment. Each product was diluted to several levels and tested against the Psa broth. The product dilution concentrations selected were based on label recommendations or on previous KVH or VLS work with Psa. The level of Psa in the broth was quantified in cfu/ml prior to use and incubated for 48 hours at 25±2°C post inoculation. After incubation, tubes were sub-cultured onto Kings B media, incubated for a further 48 hours and any observed growth of Psa V was characterised.

4.3 Minimum Inhibitory Concentration (MIC) in 0.85 % normal saline

A suspension of Psa was made in 0.85% normal saline and quantified in cfu/ml. The dilution test was repeated using a similar or narrower dilution range based on the outcome of the broth dilution test. This assay measures the activity of the chemical agent against Psa without the interference of the broth ingredients. Again each combination was incubated for 48 hours at 25 ±2°C post inoculation after which the tubes were sub-cultured onto Kings B media, incubated for a further 48 hours and the growth of Psa V was characterised.

Based on the results obtained in the ***combined*** dilution tests, a working concentration of each product was determined for use in the second part of the project.

4.4 pH sensitivity

Products were tested at progressively lower pH levels by slowly adding 0.1M HCl or a phosphate buffer to each product until the desired levels were reached. The tubes were then incubated for 48 hours at 25 ±2°C. At the end of incubation, the tubes were sub-cultured onto Kings B plates and incubated for 48 hours and growth of Psa V was characterised. This simple approach has limitations e.g. for products which are buffered acids, it is not reasonable since the addition of acid will simply prevent the dissociation of the acid product. The aim of the test was to show the correlation of pH sensitivity to products' efficacy.

4.5 Organic matter sensitivity

In order to test the sensitivity of each product to a quantifiable level of organic matter 0.1% tannic acid was used to create the working concentrations of each product (rather than water) in solution with concentrations of 1, 5, 10 and 20ppm organic matter. The mixture was then tested as per the routine procedure against Psa V: tubes were incubated for 48 hours at 25 ±2°C and then sub-cultured onto Kings B plates and incubated for 48 hours and growth of Psa V characterised.

4.6 Disc spiking

20 µl of 1 x 10^8 cfu/ml Psa V suspension in 0.85% saline was inoculated onto 8 discs of each surface (wood, steel, rubber and plastic). The discs were allowed to air dry for no more than 1 hour at ambient temperature (≈20ºC). Psa viability was tested by subsequent incubation before application of any disinfectant as a positive control (Results in Table 8).

4.7 Spray and dip applications

Each product was made up to its working concentration (Table 5) prior to spray and dip applications. For each product and material, the prepared spiked discs were either sprayed or soaked for 10s, 30s, 1 minute and 2 minutes in the test products. Following this test the discs were carefully swabbed with a sterile pre-moistened swab. The swab was then immersed into 1 ml 0.85 % saline and plated onto both 1 x Psa V media and 1 x Kings B media. The procedure was repeated for all spray and dip treatments and for each material type. These plates were incubated at 25°C ± 2°C for 2 days before checking for growth of Psa V. A set of discs of each material was spiked without any treatment and swabbed and plated as a positive control.

4.8 Scoring

After incubations, all plates were read and each plate was scored using a zero to 4 scale where 4 corresponds to abundant growth of Psa and zero is no growth as shown in Figure 1 and Table 2.

Score	Description
NG	No growth
G1	Less or equal to 25 % growth
G2	50 % growth
G3	75% growth
G4	100 % growth

Table 2: Scoring of plates

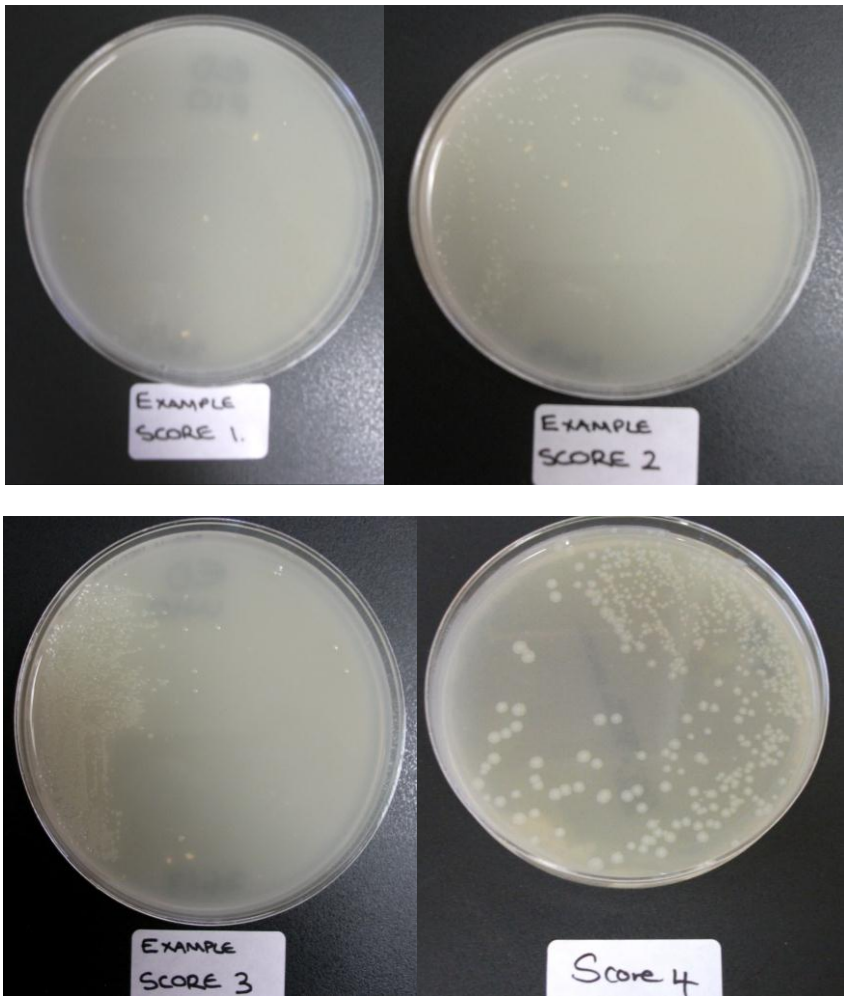

Figure 1: Scoring of Psa Colonies on Psa V Media

5.0 Results

Throughout this report we have adopted the following protocols:

- G stands for Growth of Psa bacteria and in tables has a red background
- NG stands for No-Growth of Psa bacteria and has a yellow background

5.1 MIC in broth dilution test

Prior information was available for some products (Table 3a) which focused our selection of concentrations. In some instances these did **not** result in a range of concentrations where both growth and no-growth was observed. In these cases both the broth and saline results were compared and a conservative concentration was selected given the available data. For example in the case of Envirosan broth showed no growth from 0.1% through to 1% however in saline (Table 4) we saw growth at 0.1, 0.5 and 0.8%, consequently 1% was used as the working concentration.

The *quantification* columns give the Psa concentrations recovered from each broth immediately prior to the addition of the disinfectant products.

Product	Quantification (cfu/ml)	Psa in broth			
		0.1%	0.5%	0.8%	1.0%
Envirosan	3×10^7	NG	NG	NG	NG
Trigene	3×10^7	NG	NG	NG	NG
Citrox	1×10^7	G	G	G	NG
Janola	1×10^7	G	G	G	NG
Virkon	1×10^7	G	G	G	G

Table 3a: MIC results from broth dilution. These products had prior information which meant we examined only a tight concentration range

Product	Quantification (cfu/ml)	Psa in broth			
		1%	3%	5%	10%
Zoono	1×10^7	G	G	G	G
Analyte	1×10^7	G	G	G	G
Hydrogen peroxide	1×10^7	NG	NG	NG	NG
Teraceptic	1×10^7	NG	NG	NG	NG
Kiwilustre	1×10^7	NG	NG	NG	NG
Citric acid	1×10^7	NG	NG	NG	NG
Extinct pure	1×10^7	NG	NG	NG	NG

Table 3b: MIC results from broth dilution. These products had no prior information so they were tested over a wider concentration range.

5.2 MIC in 0.85 % normal saline

This procedure for *in-vitro* efficacy in saline follows that used for broth.

Product	Quantification (cfu/ml)	Psa suspension in 0.85% saline			
		0.1%	0.5%	0.8%	1.0%
Envirosan	3×10^7	G	G	G	NG
Trigene	3×10^7	G	NG	NG	NG
Citrox	1×10^7	G	G	NG	NG
Janola	1×10^7	G	G	G	NG
Virkon	1×10^7	G	G	G	NG
Hydrogen peroxide	1×10^7	G	G	G	G
Teraceptic	1×10^7	G	NG	NG	NG
Kiwilustre	1×10^7	NG	NG	NG	NG
Citric acid	1×10^7	G	G	G	G
Extinct pure	1×10^7	NG	NG	NG	NG

Table 4a: MIC in 0.85 % saline suspension

Product	Quantification (cfu/ml)	Psa suspension in 0.85% saline			
		1%	3%	5%	10%
Zoono	1×10^7	G	G	G	G
Analyte	1×10^7	G	G	G	G

Table 4b: MIC in 0.85 % saline suspension

5.3 Determination of working concentrations from MIC studies

Despite having prior information indicating appropriate concentration ranges we sometimes found growth or no-growth persisting over our entire selected concentration range. This was unexpected but we then selected subsequent concentrations at the appropriate end of the concentrations used from the data we had.

- If results differed between broths and saline we made the conservative choice i.e. we selected the higher concentration as the working level.

- In the cases of Zoono and Analyte no efficacy was found at 10% which was the highest concentration used.

- In some cases, the product showed efficacy in the absence of broth and a working concentration was obtained (e.g. Virkon did not show any efficacy in broth at 1 % but showed efficacy at the same concentration in saline). We expect broth to affect product efficacy – probably by reducing it. While broth chemistry may be relatively poorly defined it certainly contains lots of complex organic matter and consequently has the likelihood of chemically reacting with the disinfectant products. We included this test since, along with the OM test, it may provide closer to a "real world" estimate of efficacy in the presence of reasonably heavy organic matter contamination from the orchard.

Product	Working conc
Envirosan	1%
Trigene	1%
Citrox	1%
Janola	1%
Virkon	1%
Hydrogen peroxide	3%
Teracep	1%
Kiwilustre	1%
Citric acid	3%
Extinct pure	1%
Analyte	No efficacy
Zoono	No efficacy

Table 5: Working concentration of products derived from the broth *in-vitro* results.

5.4 Effect of pH on efficacy of products

We attempted to adjust the pH of the product solution to achieve 3 different pH levels from the range 4, 5, 6, 7, 8 depending on the natural pH of the product. A fourth undisturbed treatment was left as a control. Measured pH levels are recorded since small volumes and different buffering meant that final pH levels varied. Each product was tested at its working concentration determined from the earlier MIC tests.

- *Actual pH* refers to the unadjusted solution pH
- *Adjusted pH* is what was achieved by adding small amounts of HCl or phosphate buffer (to increase pH).

1 % Envirosan	Actual pH	Adjusted pH	Growth
1	6.9	4.1	NG
2	6.9	5.6	NG
3	6.9	8	G
4	6.9	N/A	NG

Table 6(a) Natural pH very mildly acidic, no efficacy when pH was raised (basic solution)

1 % Trigene	Actual pH	Adjusted pH	Growth
1	7.3	4.5	NG
2	7.3	6.1	NG
3	7.3	8.1	NG
4	7.3	N/A	NG

Table 6(b) Natural pH mildly basic, no pH sensitivity

1 % Citrox	Actual pH	Adjusted pH	Growth
1	6.4	4.6	NG
2	6.4	5	NG
3	6.4	7	G
4	6.4	N/A	NG

Table 6(c) Natural pH mildly acidic, no efficacy when pH was raised (basic solution).

1% Janola	Actual pH	Adjusted pH	Growth
1	8.4	4	NG
2	8.4	5.8	NG
3	8.4	7	NG
4	8.4	N/A	NG

Table 6(d) Natural pH basic, no pH sensitivity.

1% Virkon	Actual pH	Adjusted pH	Growth
1	4	5.1	NG
2	4	6.5	NG
3	4	7	NG
4	4	N/A	NG

Table 6(e) Natural pH strongly acidic, no pH sensitivity.

3 % Hydrogen peroxide	Actual pH	Adjusted pH	Growth
1	6.8	4	NG
2	6.8	5	NG
3	6.8	8	NG
4	6.8	N/A	NG

Table 6(f) Natural pH very mildly acidic no pH sensitivity.

1 % Teracep	Actual pH	Adjusted pH	Growth
1	4.8	4	NG
2	4.8	6.2	NG
3	4.8	7.1	NG
4	4.8	N/A	NG

Table 6(g) Natural pH reasonably strongly acidic no pH sensitivity.

1% Kiwilustre	Actual pH	Adjusted pH	Growth
1	4.1	5.1	G
2	4.1	6.1	G
3	4.1	7.6	G
4	4.1	N/A	NG

Table 6(h) Natural pH strongly acidic, shows pH sensitivity (no control) when it is raised.

3 % Citric acid	Actual pH	Adjusted pH	Growth
1	2.5	3	G
2	2.5	4	G
3	2.5	5	G
4	2.5	N/A	NG

Table 6(i) Natural pH very strongly acidic shows pH sensitivity (no control) when it is raised.

Extinct pure	Actual pH	Adjusted pH	Growth
1	4	5.6	NG
2	4	6	NG
3	4	7	NG
4	4	N/A	NG

Table 6(j) Extinct pure Natural pH strongly acidic no pH sensitivity.

10% Analyte	Actual pH	Adjusted pH	Growth
1	7.3	4	G
2	7.3	6	G
3	7.3	8.3	G
4	7.3	N/A	G

Table 6(k) Natural pH mildly basic. This product showed no pH sensitivity however; consistent with the broth results it also showed no Psa control.

10% Zoono	Actual pH	Adjusted pH	Growth
1	7.03	4.1	G
2	7.03	5	NG
3	7.03	6	G
4	7.03	N/A	G

Table 6(l) Zoono had neutral pH and, consistent with the broth results, showed no Psa control except at pH = 5. Given the growth observed at pH 4 and 6 this observation seems to be an outlier.

5.4.1 Comments

This is not a chemistry report so we are not in a position to explain all the pH results in chemical terms. We can make some general comments:

We expected this pH sensitivity test to be inappropriate for some products. Any product which gains its efficacy by pH *alone* will surely have it changed when the pH is *forced*

to a different level e.g. perhaps citric acid. While we used final pH as an indicator for this simple test we might also have explored the volume of H^+ required forcing the pH change (i.e. as an indication of resiliency of buffering) but this is simply one of the many further explorations possible.

- Other products such as chlorine dioxide (Extinct pure in these tests) which may rely on *in-solution* chemistry (including pH) to create their active ingredient *in-situ* may also be intrinsically changed by changing pH. In the case of chlorine dioxide it did not appear to affect efficacy.

- Zoono and Analyte showed no efficacy in this test – as we expected from their broth results.

5.5 Effect of organic matter on efficacy of products

Again the exploration of sensitivity to organic matter could be extensive. Earlier work (VLS report "Efficacy study on Envirosan, Trifilm, Citrox PWT and Trigene used as disinfecting products on field tools (Secateurs)" showed that the presence of wood debris on secateurs affected the efficacy of disinfecting. This examination is an attempt to indicate any intrinsic sensitivity of the product to the presence of organic matter using a simple, but repeatable, test. Each product was tested at its *working* concentration determined from the earlier MIC tests.

Product	Prod Conc^n	Concentration of organic matter			
		20 ppm	10 ppm	5 ppm	1 ppm
Envirosan	1%	NG	NG	NG	NG
Trigene	1%	NG	NG	NG	NG
Citrox	1%	NG	NG	NG	NG
Janola	1%	G	G	G	G
Virkon	1%	G	G	G	G
Zoono	10%	G	G	G	G
Analyte	10%	G	G	G	G
Hydrogen peroxide	10%	NG	NG	NG	NG
Teracep	1%	G	NG	NG	NG
Kiwilustre	1%	NG	NG	NG	NG
Citric acid	10%	NG	NG	NG	NG
Extinct pure	1%	NG	NG	NG	NG

Table 7: Effect of organic matter on efficacy of products. The concentration levels (20-1ppm) correspond to different concentrations of organic matter used in making up the test solution of product.

5.5.1 Comments

We expected that any product which reacted in a purely chemical way with organic matter might change its efficacy against PsaV. In simple terms the organic matter might simply react with the entire active ingredient leaving none to react with Psa V. We realise the outcome in these circumstances may depend on the *level* of organic matter present however it was felt that an attempt to explore this possibility was better than ignoring it. In retrospect a further useful test would have been to apply an "overwhelming" level of organic matter representing wood or soil contamination.

5.6 Quantification of Psa V used for spiking

Several batches of Psa V were required for spiking the many discs used for testing efficacy of the products on each surface by both dipping and spraying. Each batch was quantified by undertaking a serial dilution followed by manual counting of colonies on plates. Following the procedure described earlier the levels of Psa V deposited and surviving on each disc type were quantified by swabbing, replating, incubating and scoring. Results are shown in Table 7. Clearly the procedures retain a highly surviving population of Psa.

Material	Quantification of recovered Psa (cfu/ml)
Wood	4×10^8
Plastic	5×10^9
Rubber	9×10^9
steel	4×10^9

Table 8: Quantification of Psa on discs after inoculation on disc

5.7 Spraying and Dipping Efficacy

A table of results is presented for each product at its working concentration showing efficacy on each surface for each application method (in rows) and for each media type and exposure duration (in columns). Where colony growth was found it was tested for Psa using PCR and number of amplification cycles required to reach threshold fluorescence (C_q) is given. If more than one application duration resulted in colony growth the plate indicated by an asterisk corresponds to the PCR score given in the confirmatory column.

Envirosan: 1%		Psa-V media					Kings B media				
Test Material	Applic^n Method	10 sec	30 sec	1 min	2 min	PCR Colony confirm^n	10 sec	30 sec	1 min	2 min	PCR Colony confirm^n
Wood	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
Plastic	Spray	G2*	G1	NG	NG	18.62*	G2*	G1	NG	NG	19.74*
	Dip	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Tyre	Spray	G1*	NG	G2	G1	18.51*	G1*	NG	G2	G1	18.07*
	Dip	G3*	G1	G2	NG	18.13*	G3*	G1	G3	NG	17.95*
Metal	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A

Table 9a: Envirosan results.

Envirosan showed consistent efficacy (NG: no-growth) at 10 seconds and longer contact times on both wood and metal. Since 10s is the shortest time used in the trial this indicates the maximum efficacy we could discover for both spray and dip applications on these surfaces. By contrast it did ***not*** appear to be a suitable disinfectant for tyre sanitisation. While it showed complete efficacy on plastic when dipped it showed consistent growth (i.e. not effective sanitisation) on plastic at times less than a minute when sprayed.

Trigene: 1%		Psa-V media					Kings B media				
Test Material	Applic^{n} Method	10 sec	30 sec	1 min	2 min	PCR Colony confirm^{n}	10 sec	30 sec	1 min	2 min	PCR Colony confirm^{n}
Wood	Spray	G1*	NG	NG	NG	19.19*	G1*	NG	NG	NG	29.18*
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
Plastic	Spray	G3*	G2	G2	G2	15.29*	G3*	G3	G3	G2	17.01*
	Dip	NG	NG	NG	NG	N/A	G1*	G1	NG	NG	22.92*
Tyre	Spray	G3*	G2	G2	G2	15.83*	G3*	G3	G3	G3	16.47*
	Dip	G3*	G3	G2	G3	15.48*	G3*	G3	G2	G3	16.9*
Metal	Spray	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
	Dip	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

Table 9b: Trigene

Trigene showed good efficacy for sanitisation of metal surface at 10 seconds in both application methods and 10 and 30 seconds in dip and spray application respectively on wood.

Citrox: 1%		Psa-V media					Kings B media				
Test Material	Applic^{n} Method	10 sec	30 sec	1 min	2 min	PCR Colony confirm^{n}	10 sec	30 sec	1 min	2 min	PCR Colony confirm^{n}
Wood	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
Plastic	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	G1*	G1	NG	NG	16.85
Tyre	Spray	G1	G1	G1*	G2	18.37	G1	G1*	G1	G2	25.86
	Dip	G2*	G2	G2	G1	15.21	G3*	G2	G3	G1	16.04
Metal	Spray	NG	NG	NG	NG	N/A	G1*	G1	G1	NG	28.14
	Dip	NG	NG	NG	NG	N/A	G1*	G1	G1	NG	18.37

Table 9c: Citrox

Citrox showed efficacy on wood at 10 seconds for both application methods. It is not recommended for sanitisation on tyres or metal surfaces and showed some growth at 10s dipping on plastic and Kings B. This last result was confirmed as Psa V (i.e. it was not something else growing on the broad spectrum media) but it may be treated with a little surprise since the spray application (which we might generally expect to be less effective than dipping) showed efficacy at the same time (10s).

Janola: 1%		Psa-V media					Kings B media				
Test Material	Applic^{n} Method	10 sec	30 sec	1 min	2 min	PCR Colony confirm^{n}	10 sec	30 sec	1 min	2 min	PCR Colony confirm^{n}
Wood	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
Plastic	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
Tyre	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
Metal	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A

Table 9d: Janola

Janola showed efficacy on all surfaces at 10 second in both dip and spray application. Unfortunately it is not particularly friendly to unprotected skin.

Virkon: 1%		Psa-V media					Kings B media				
Test Material	Applic^{n} Method	10 sec	30 sec	1 min	2 min	PCR Colony confirm^{n}	10 sec	30 sec	1 min	2 min	PCR Colony confirm^{n}
Wood	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
Plastic	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	G1*	NG	NG	NG	24.85*
Tyre	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
Metal	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A

Table 9e: Virkon

Virkon showed efficacy on all surfaces for both application methods at 10 seconds except for the dip application for 10sec. This was the same "outlier" combination (10s, Kings B and Dipping) seen for citrox above and the same comments apply.

Hyd Peroxide: 3%		Psa-V media					Kings B media				
Test Material	Applic^{n} Method	10 sec	30 sec	1 min	2 min	PCR Colony confirm^{n}	10 sec	30 sec	1 min	2 min	PCR Colony confirm^{n}
Wood	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
Plastic	Spray	G1	G1*	G2	NG	15.18	G1*	G2	G3	G1	20.24
	Dip	G3*	G3	G3	NG	20.24	G3*	G4	G3	NG	16.8
Tyre	Spray	G1	G1	G2*	NG	17.37	G1	G1	G2*	NG	16.57
	Dip	G2*	G3	G1	G1	19.5	G3*	G3	G1	G2	15.56
Metal	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A

Table 9f: Hydrogen peroxide

Hydrogen peroxide worked well on wood and metal but not plastic or rubber. In those cases it showed some efficacy at the maximum contact duration of 2min. It may be worth looking at a higher concentration of this product which is relatively cheap and "uncomplicated".

Teracep: 1%		Psa-V media					Kings B media				
Test Material	Applic^{n} Method	10 sec	30 sec	1 min	2 min	PCR Colony confirm^{n}	10 sec	30 sec	1 min	2 min	PCR Colony confirm^{n}
Wood	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
Plastic	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	G2*	NG	NG	NG	22.01*
Tyre	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	G3*	NG	NG	NG	18.37	G3*	NG	NG	NG	20.08*
Metal	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A

Table 9g: Teracep

Generally effective however it allowed growth at 10s on both media for dipping on tyres and plastic (on Kings B).

Kiwilustre: 1%		Psa-V media					Kings B media				
Test Material	Applic^{n} Method	10 sec	30 sec	1 min	2 min	PCR Colony confirm^{n}	10 sec	30 sec	1 min	2 min	PCR Colony confirm^{n}
Wood	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
Plastic	Spray	NG	NG	NG	G1*	22.26*	NG	NG	NG	G1*	24.9*
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
Tyre	Spray	NG	NG	NG	G1*	17.87*	NG	NG	G4*	G1	25.92*
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
Metal	Spray	NG	NG	NG	NG	N/A	G1*	NG	NG	NG	26.63*
	Dip	NG	NG	NG	NG	N/A	NG	NG	G1*	NG	24.54*

Table 9h: Kiwilustre. Apparent outliers in result rows 3, 5 and 8.

This is the first test which clearly has outliers. In the case of plastic, spray, 2min; tyre, spray, 2min and metal, dip, 1 min we see the same combinations being effective at shorter times. One of the reasons why we used different people at different times to undertake the tests is to minimise possible cause of such outliers. Clearly it would be good to repeat these tests however they currently stand as outliers.

Extinct Pure: 1%		Psa-V media					Kings B media				
Test Material	Applic^{n} Method	10 sec	30 sec	1 min	2 min	PCR Colony confirm^{n}	10 sec	30 sec	1 min	2 min	PCR Colony confirm^{n}
Wood	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
Plastic	Spray	G3*	G3	G3	NG	18.21*	G3	G3	G3*	NG	20.36*
	Dip	NG	NG	G1*	NG	16.89*	G1	G2	G1*	NG	21.43*
Tyre	Spray	G1	G2	G2*	G2	22.54*	G1	G2*	G2	G3	25.02*
	Dip	G1	G1*	G1	G3	21.59*	G3	G1	G1*	G2*	22.37*
Metal	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A

Table 9i: Extinct pure Apparent outlier in row 4

Again there is one case (plastic, dip, 1min) which is surrounded by effective disinfecting which we label an outlier.

Citric: 3%		Psa-V media					Kings B media				
Test Material	Applic^{n} Method	10 sec	30 sec	1 min	2 min	PCR Colony confirm^{n}	10 sec	30 sec	1 min	2 min	PCR Colony confirm^{n}
Wood	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
Plastic	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
Tyre	Spray	NG	NG	NG	NG	N/A	G*	NG	NG	NG	*not Psa
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
Metal	Spray	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A
	Dip	NG	NG	NG	NG	N/A	NG	NG	NG	NG	N/A

Table 9j: Citric acid

This is the only case in the entire project (shown in green) where we found growth which was not verified as PsaV.

6.0 Summary

A brief summary of the characteristics and results for each product tested is shown in Table 10. Solution pH, and the likelihood of a residue together with the results from the pH and organic matter sensitivity tests are all tabulated along with the *worst* performance in the surface disinfectant tests. The time given in this section is the minimum time required to disinfect the surface by the method shown. Many products have times of 10s – remember this was the shortest time we tested so the products' performance may be better than this.

We were surprised by the performance of some products in the MIC tests where we did not find a "boundary" where no-growth changed to growth – in these instances further work could usefully be undertaken to determine the lowest effective concentration of the product.

The approaches used to try and assess pH and organic matter sensitivities were developed for this project. They could undoubtedly be improved with further work but are provided as an indication to guide users in situations when either pH might affect utility (such as when applied to fruit) or when the surface to be cleaned may be contaminated by organic matter. Future work could consider testing in the presence of an "overwhelming" level of organic matter since I am surprised that a product based on chlorine dioxide showed no sensitivity to organic matter (at the levels we used) whereas we might expect this oxidising agent to be "used up" in the presence of enough organic matter. Comparison of the saline and broth MIC test results also provides an indication where efficacy may be changed by the presence of organic matter.

In the surface type-by-application-method part of the project a number of outliers were recorded. We defined an outlier in this context as an occasion when lower concentrations or longer times had resulted in no-growth. Again these instances should be re-examined. Two products, Zoono and Analyte, showed no efficacy at any of the concentrations we tested in the MIC tests so were not included in the surface/application method part of the trial

Summary				Sensitive to...		Spray efficacy				Dip efficacy			
Product tested	Conc^{n}	pH	Likely Residue	pH	OM	Wood	Plastic	Tyre	Metal	Wood	Plastic	Tyre	metal
Envirosan	1%	6.9	Yes	B	NS	10s	1 min	NE	10s	10s	10s	NE	10s
Trigene	1%	7.3	Yes	NS	NS	30s	NE	NE	10s	10s	NE	NE	10s
Citrox	1%	6.4	No	NS	NS	10s	10s	NE	2 min	10s	1min	NE	2min
Janola	1%	8.4	No	NS	S	10s	10s	10s	10s	10s	10s	10s	10s
Virkon	1%	4	Yes	NS	S	10s	10s	10s	10s	10s	30s	10s	10s
H_2O_2	3%	6.8	No	NS	NS	10s	NE	2min	10s	10s	2min	NE	10s
Teracep	1%	4.8	No	NS	S	10s	10s	10s	10s	10s	30s	3s	10s
Kiwilustre	1%	4.1	No	S	NS	10s	NE	NE	30s	10s	10s	10s	2min
Extinct pure	1%	4	No	NS	NS	10s	2min	NE	10s	10s	2min	NE	10s
Citric acid	3%	2.5	No	S	NS	10s	10s	30S	10s	10s	10s	10s	10s
Zoono	10%	7.03	Yes	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Analyte	10%	7.3	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Abbrevs: NE = Not Effective; NS = Not sensitive; S = Sensitive, B=sensitive to *basic* conditions;

Table 10 Summary of results. Times shown are the minimum required for disinfecting i.e. short (10s) is best.

7.0 Acknowledgements

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