Efficacy of Oxyspray on PSA-V on Hort16A Kiwifruit Leaves

Confidential Report Prepared For: ZESPRI International Limited

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Westhaven Orchard

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1.0 Objective

The purpose of this trial was to investigate the efficacy of the stabilized chlorine dioxide product Oxyspray 3000 for reduction in PSA-V inoculum, on Hort16A kiwifruit leaves, using a standard orchard sprayer.

2.0 Site Details

Table 1: Trial Site Details

Location	Westhaven, 649 Rangiuru Road, Te Puke KPIN 7618
Contacts	Owners: RJ West & K West Family Trust Partnership, 07 5700167, 027 5335 037 Trial Manager: Lynda Hawes, 07 5430848, 027 4824441 Email lynda.hawes@xtra.co.nz Post Harvest Facility: EastPack Ltd, Te Puke
Site Details	Mature Hort 16 A confirmed PSA-V positive, showing primary and secondary symptoms
Plot Size	Entire block, rows 40 - 48, except last 28 bays as untreated control area. Vines, 3.5m row width, 6.0 m bay m length 10 sample sites per treatment
Water rates	1000 litres/ha
Application	Treatment as below
Sprayer	Typhoon Cropliner 2000

3.0 Treatments/ Replicates/Plot Number

Treatments were applied in a non randomised manner in the orchard block occupied by rows 40-48. Treatment 1 was Oxyspray applied once at 50ppm and Treatment 2 was an untreated control.

Treatment 1 comprised the balance of the block, which was sprayed with Oxyspray at 50ppm. Sample sites were selected within this treated area, close to the untreated area, but where spray coverage was normal, for each set of leaf samples collected.

Treatment 2 was untreated control vines, comprising a marked area across the east end of the block. In addition, the equivalent area in the first two rows of both blocks adjacent to the trial block was also not sprayed, to avoid any possibility of overspray or drift onto untreated control vines.

Refer Appendix 9.1 for Orchard Map

4.0 Product, Rates and Treatments Times

The orchard is already infected with PSA-V. Treatment 1 was applied once using the proprietor recommended rate and standard orchard equipment.

Table 2: Application Rates

Product	Rate	Adjuvant
Untreated	-	-
Oxyspray 3000	15L/1000L/ha at 50ppm	Spreadwett 250ml/100L

Note that the rate of 50ppm Oxyspray is presently greater than that permitted by ACVM.

Treatments were measured on site using an appropriate facility and from newly opened containers. The applicator holds a current GrowSafe certificate.

Application was at 1000l/ha equivalent, wetting all foliage. Application was made in good drying conditions and at the beginning of a weather window which had several days of dry weather post application.

Table 3: Application Details

Date	Treatment	Time	Growth Stage	Applicator	Temperature	Wind Speed and Direction
13/03/12	Oxyspray 3000 at 50ppm	11.45 am – 12.00 midday	Mature canopy	Peter West	21^{0}C	11 – 12 km/hour; ESE

Rainfall data from the nearest meteorological station was recorded, over the duration of the trial period. **Refer Appendix 9.2 for Rainfall Data**

5.0 Assessments

Water Quality

The proprietor, Scitex New Zealand Ltd arranged for a spray water sample to be collected just prior to mixing to check water quality.

Spray Mix Concentration

The proprietors arranged for collection of a sample of the spray from the spray tank, and from the nozzles (if possible), to assay for chlorine dioxide concentration at NZ Laboratories, Ruakura, Hamilton.

Spray Coverage

The proprietors arranged for assessment of spray coverage using water sensitive papers at row centre and leader locations in canopy above the wire.

Inoculum Reduction

Leaf samples were collected before, immediately after spray application had dried, and were proposed to be collected on days 1,2,4,6,8,10 and 12 after application.

Each sample was one mature leaf, with light PSA type spotting on the leaf.

Figure 1: Typical Bagged Leaf Sample

Leaf sample position was from above the canopy wires and no closer to the leader than 1 wire out.

Results were reported within two days of sampling. As leaf sample results were reported, it became apparent Oxyspray was not having a very persistent effect. The decision was taken in consultation with all parties, to discontinue sample collection. Day 6 samples after application were the final samples.

Each leaf sample had light PSA type leaf spotting present, so that the laboratory testing could ascertain the treatment effect on surface inoculum.

Table 4: Sample Numbers and Timing (1 leaf = 1 sample)

Day	Sample type	No. samples per type	Total Number of Samples Collected
Pre application	Treated, Control untreated	10,10	20
Post application, immediately when dry	Treated	10	10
+1	Treated, Control untreated	10,10	20
+2	Treated, Control untreated	10,10	20
+4	Treated, Control untreated	10,10	20
+6	Treated, Control untreated	10,10	20

Each leaf was picked and placed into a clean Ziploc® bag, with disposable gloves used by the sample collector and disposed of between each sample.

The labelled bags were delivered on the same day as the sample was collected to Verified Laboratory Services.

Verified Laboratory Services undertook leaf disk extraction and culturing on

- A specific PSA-V semi selective medium to determine presence or absence of viable PSA-V bacteria and a rating for the amount of viable bacteria, if present
- A broad spectrum bacterial medium, to determine presence or absence of other viable bacteria on Kings B agar medium

The testing procedure extracted from the leaf surfaces, not from within leaf tissue.

The samples were processed as per standard operating procedure at VLS and streaked aseptically onto 1 x PSA-V-V media and 1 x Kings B media and incubated at 25°C± for 2 days.

6.0 Results

Water Quality

Water quality was tested and reported by NZ Labs, Hamilton. **Refer Appendix 9.3 attached.**

The water sample collected was found to have a Heterotrophic Plate Count of 52,000cfu/100ml.

Spray Mix Concentration

Tank mix concentration was tested, but a nozzle output sample was not able to be collected for testing. Testing was carried out by NZ Labs, Hamilton. **Refer Appendix 9.4 attached.**

The report shows the first tank mix sample (Oxyspray 1) to have had a higher concentration of chlorine dioxide at 65ppm than the targeted rate of 50ppm.

Figure 2: Mid Row Spray Coverage

Spray Coverage

Water sensitive papers observed in the middle of the canopy, above the wire, were completely blue indicating 100% spray coverage with no gaps in spray cover.

Inoculum Reduction

After incubation, three separate measures were reported for each sample.

Firstly, the plates were read and growth of PSA-V like colonies were identified based on morphological characteristics on selective PSA-V - V media. The first result is a presence or absence of PSA-V reported as growth (G) or no growth (NG).

Secondly, the plates were given a PSA-V growth score on a scale of 0 – 4, where 0 is no growth and 4 is 100 % growth throughout the plate.

Thirdly, the presence or absence of other bacteria was also determined from the Kings B broad spectrum media and reported as growth (G) or no growth (NG).

Results of the lab reading are shown in Table 5. None of the results is statistically significant although there appears to be a possible reduction directly after and one day after application.

The results are presented in Table 5 and Figures 3 – 5.

Table 5: Inoculum Reduction

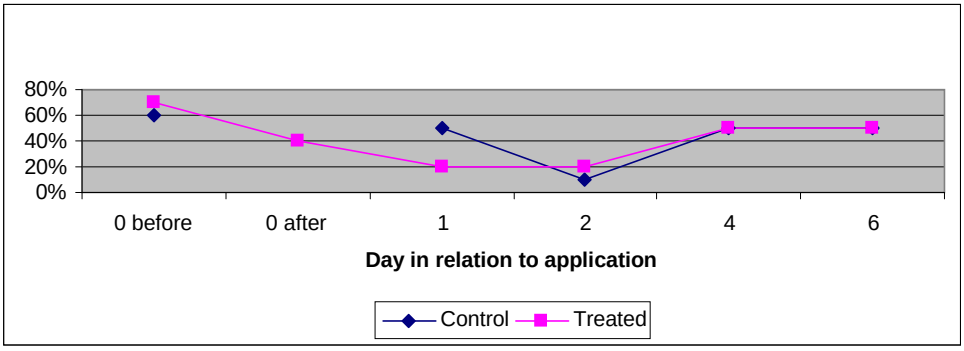
Control untreated	Percentage of samples with Psa-V like colonies		Average Psa Growth Score (and ranges)		Percentage of samples with other bacterial colonies	
day	Control	Treated	Control	Treated	Control	Treated
0 (before application)	60%	70%	1.00 (0 – 3)	0.80 (0 – 2)	60%	70%
0 (after application)		40%		0.50 (0 – 2)		40%
+ 1	50%	20%	0.60 (0 – 2)	0.20 (0 – 1)	80%	70%
+ 2	10%	20%	0.10 (0 – 1)	0.40 (0 – 3)	70%	100%
+ 4	50%	50%	0.50 (0 – 2)	0.50 (0 – 1)	100%	100%
+ 6	50%	50%	0.50 (0 – 1)	0.80 (0 – 2)	90%	100%

The results show that before treatment, PSA-V was present on 60 % of the leaves on control vines and 70% of leaves on vines to be treated.

As soon as spray dried after application, PSA-V was present on 40 % of the sampled leaves on treated vines.

One day after application, PSA-V was present on treated leaves at a lower level (20%) than on control leaves (50%).
Two days after application, PSA-V was present on treated leaves at a higher level (20%) than on control leaves (10%) Both results were considerably lower than at the start of the trial prior to application, two days earlier.

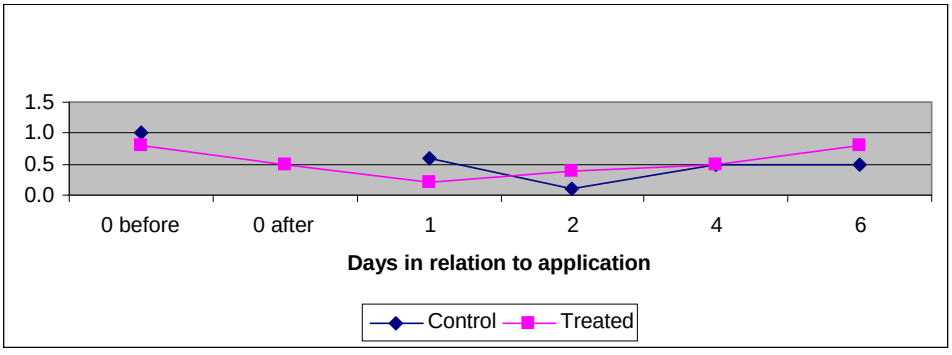
Figure 3: PSA-V like Colonies Average Presence



From the fourth day after application, PSA-V was present at the same level on both treated and control leaves (50%).

The PSA-V growth score averages showed a similar pattern as for Presence of PSA-V like colonies, in that for both treated and control leaves, the average growth score started at a higher level at day zero, then declined to a low level two days after application and increased again from that time.

Figure 4: PSA-V Colony Growth Score

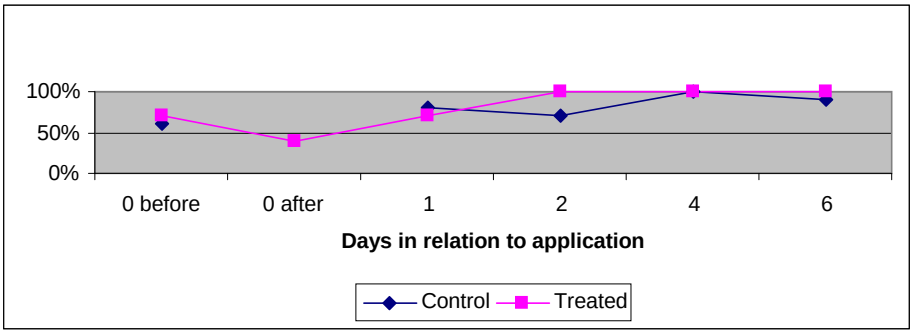


Other bacteria were detected frequently on both treated and control leaves at all sample intervals. The least number of detections was for leaves sampled immediately after Oxyspray had dried, on day zero (40%).

Other bacteria were present on Oxyspray treated leaves at 70% before treatment and 70% by 1 day after treatment, then at 100% after that time.

Other bacteria were present on control leaves at 60% before treatment and at higher levels after that time.

Figure 5: Other Bacteria Average Presence



7.0 Discussion

There was no statistically significant reduction in Psa – V inoculum or other bacteria seen in this trial, comparing treated versus untreated samples.

This trial has used a new methodology to assess Oxyspray application on PSA – V inoculum reduction.

The number of replicates per sample round may have been insufficient to show any significant difference between treated versus untreated.

The day two after application results show a large difference in both treated and control, compared with treated and control results at other sample times. This difference may be due to normal daily variation in PSA – V levels or error in technique.

The results are indicative that the methodology is worthy of further testing. The methodology might be a further step in evaluating product efficacy, beyond the in vitro agar plate testing and broth testing, greenhouse plant testing and outdoor potted plant testing.

The methodology needs refinement and greater replication.

The heterotrophic plate count at 52,000cfu/ml in the water is not unusual for untreated water sources.

The spray tank mix sample in fact showed that the actual concentration of chlorine dioxide was higher than was intended at 65 ppm. Given the very short time (a few minutes) between spray mixing and application, it seems likely that target or higher chlorine dioxide concentration was achieved at application.

The complete coverage of water sensitive paper in the zone where leaf samples were collected, confirms that such leaves collected for laboratory testing would have been similarly covered by the spray. Given the full coverage of target surfaces, as shown by the completely blue water sensitive paper, a much lower level of PSA – V and other bacteria found after treatment, might have been expected. However, we do not know how other compounds would have performed under the same conditions.

Rainfall did not occur from before application on day one until after 5 am on day 6 after application, when the final leaf samples were collected for laboratory testing. Therefore Oxyspray persistence was not affected by rain in this trial.

Oxyspray may be able to achieve some knockdown of PSA-V bacteria, but we were unable to demonstrate that in this trial.

Despite complete spray coverage on these leaves and sampling soon after application on the same day and again the next day, Oxyspray did not kill all the PSA-V and other bacteria present at those times.

The most recent PSA Product Trials Summary of Results in Vivo 16 May 2012 shows Oxyspray plus Anngro; and Oxywash, did not demonstrate efficacy in at least one cultivar at at least one timing after inoculation.

Oxyspray is listed on the current Zespri programme as an Allowed Other Compound and is Zespri approved as a fruit bin and picking bag sanitiser. I.e. for a short term effect on inert surfaces.

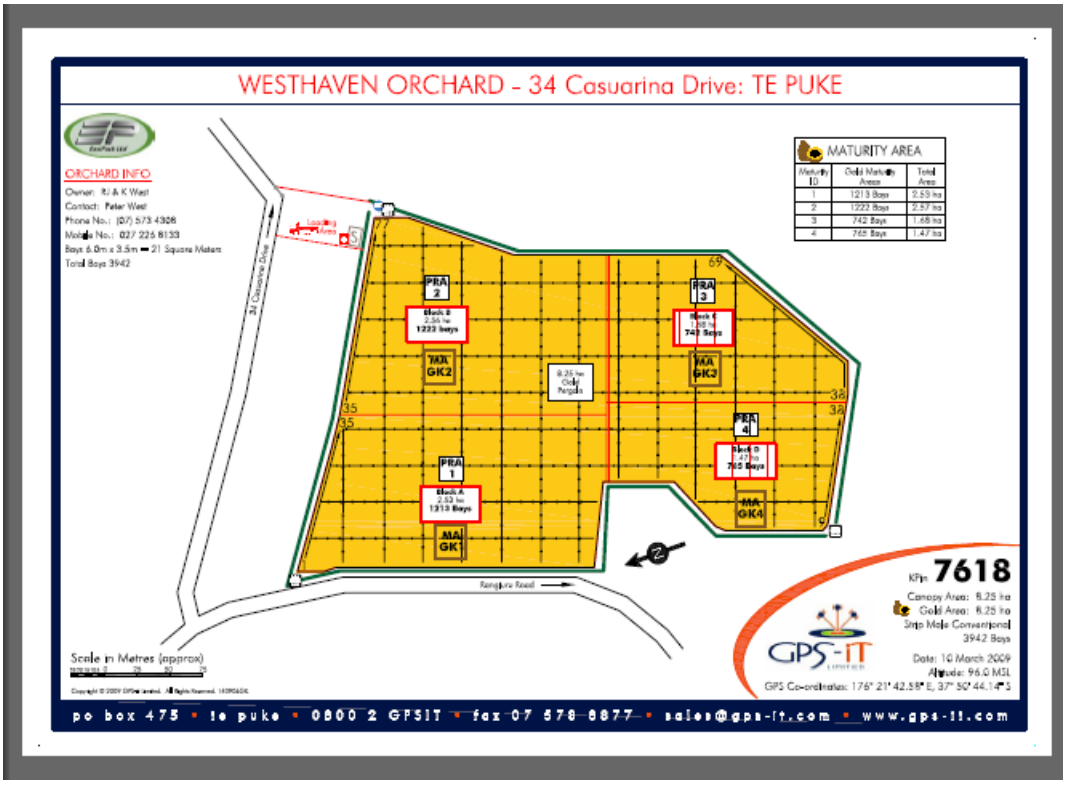
Based on our current knowledge of PSA-V infection pathways, protecting kiwifruit plants from PSA-V infection requires maintenance of protective cover, on plants, on an ongoing basis.

These findings underline the importance of understanding the threat of ongoing fresh inoculum production in a PSA-V environment. Knocking down the PSA-V inoculum level today and having no protection against newly arriving PSA-V inoculum after tomorrow, is not a sustainable production strategy.

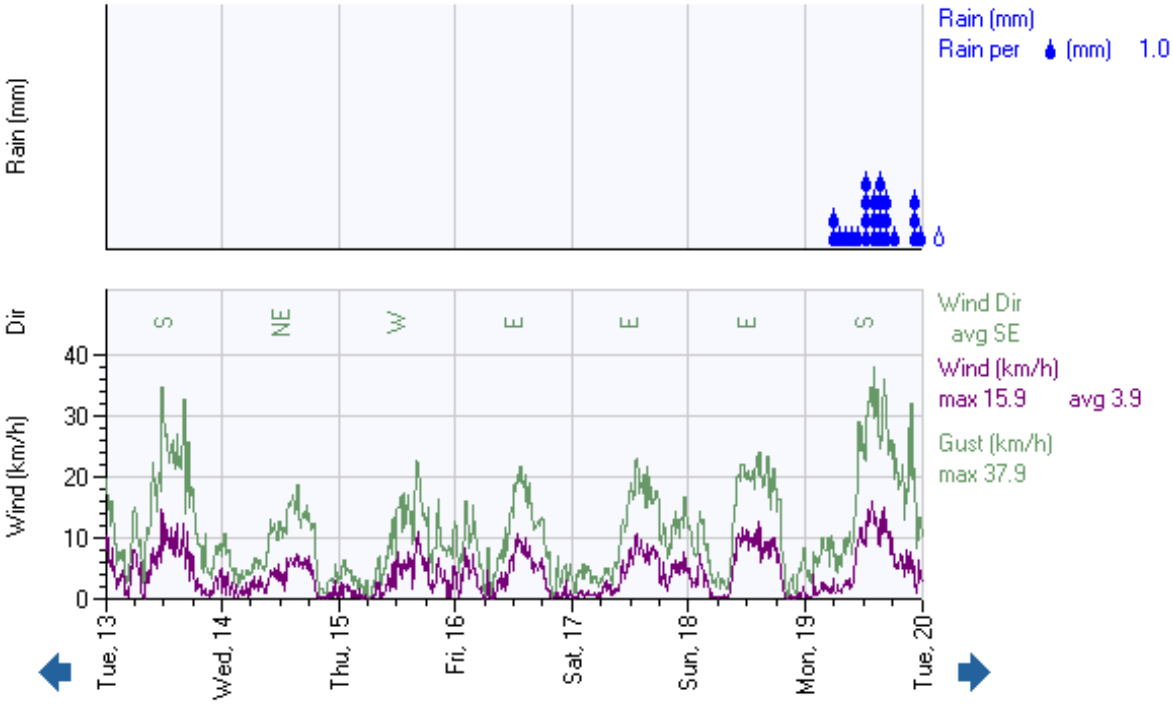
8.0 Acknowledgements

- Russell and Peter West, Westhaven Orchard

Appendix 9.1 Orchard Map



Appendix 9.2: Rainfall Data 13 – 20 March 2012, Schultz Site



Appendix 9.3 Water Lab Test Report

Farm No:	2001053
Sampled:	13-Jun-2002
Received:	14-Mar-2012
Reported:	21-Mar-2012

NZLABS

Auckland - Hamilton - Hastings - Christchurch - Dunedin

Any interpretation or recommendations are prepared independently by your consultant

Client Details
Saitex New Zealand Limited
PO Box 314
TE AWAMUTU 3840

Consultant Details
Contract Packaging & Storage Ltd

PO Box 4678
MT MAUNGANUI SOUTH 3149

Property Name Westhaven Kiwifruit Orchards

Water Lab Test Results

Sample Name	Lab SampleID	HPC Heterotrophic Plate Count cfu/100mL
Westhaven	12W03618	52,000

Test Units and Test Methods

Test	Unit	Unit Description	Test Method
HPC 35°C	cfu/100mL	Colony forming units per 100 millilitres	See attached report.

Appendix 9.4 Spray Tank Mix Concentration Report

Form No:	5011735
Sampled:	14-Mar-2012
Received:	15-Mar-2012
Reported:	16-Mar-2012

NZLABS
Auckland - Hamilton - Hastings - Christchurch - Dunedin

Any interpretation or recommendations are prepared independently by your consultant

Client Details
Scitex (New Zealand) Limited
PO Box 314
TE AWAMUTU

Consultant Details
New Zealand Laboratory Services Ltd
Ruakura Research Centre
PO Box 281
East Street
HAMILTON

Property Name Chlorine Dioxide

Test Results

Sample Name	ClO2# Chlorine Dioxide ppm	pH† Acidity / Alkalinity
Oxyspray 1	85	7.2
Oxyspray 2	80	7.4

Test Units and Test Methods

Test	Unit	Unit Description	Test Method
ClO2	ppm	mg ClO2 per kg	Iodometric Method I AWWA, 4500 Cl B
pH			Electrometric, APHA 4500_H B, 21st Ed. 2005.

†Indicates tests which are not IANZ Registered.
#Indicates Subcontracted Tests

Signed

Brent Miller: Soil & Pot Team Leader

All results reported on material AS RECEIVED unless stated otherwise.