Plant & Food RESEARCH
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PSA 5.1 Product screening test 2

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February 2011

A report prepared for

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SPTS Client Report No. 5034

PFR Client Rpt No. 42266

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PLANT & FOOD RESEARCH *Pseudomonas syringae* pv. *actinidiae* (Psa) RESEARCH NOTE

KEY PROJECT DETAILS

Project Title	*In vitro* product testing
Project Protocol No./ Objective No.	5.1 Product screening - Test 2: Growth rate
Project Leader	Kerry Everett
Research Requested / Contracted by	ZESPRI Group Ltd
Date (Month, Year)	January 2011
Based on information as at	January 2011

RESEARCH QUESTION AND AIM

Treatments to limit the spread of Psa in orchards are required. ZESPRI have requested preliminary *in vitro* testing of products for activity against *Pseudomonas syringae* pv. *actinidiae*.

Aim: To carry out in vitro tests on products for potential efficacy for control of New Zealand isolates of *P. s.* pv. *actinidiae*

METHODOLOGY (Include brief details of experimental design, methodology and protocols)

Experimental design

P. s. pv. *actinidiae* was isolated from leaf and flower tissue collected from KPIN 7668 (RP2, KEP3), and from leaf and flower tissue collected from another Bay of Plenty orchard (KPIN 2389, KEP1 and KEP2). Single cells from the isolation plates were used to generate the test cultures. Two types of tests were conducted, the first that measured the zone of inhibition of a bacterial 'lawn' around filter paper discs infiltrated with the test products, and the second measuring the growth of the bacteria in the presence of the test products. ZESPRI staff sourced and selected those products to be tested. The products evaluated are shown in Table 1.

Methods and protocols

Products to be tested were adjusted to twice the following concentrations: 1 µg/ml, 10 µg/ml, 100 µg/ml and 1000 µg/ml. A 500-ml aliquot of each of these concentrations of product was added to 1.5-ml Eppendorf tubes containing 500 ml of 2x concentration nutrient broth, to result in concentrations of 1x nutrient broth and 1x 1 µg/ml, 10 µg/ml, 100 µg/ml and 1000 µg/ml products. Biological products were adjusted to a concentration of 2x 10^5, 10^6, 10^7 and 10^8 cfu/ml before adding 500 ml to 500 ml 2x nutrient broth to result in 1x concentration of products and nutrient broth. A suspension of cultures of KEP1, KEP2 and KEP3 that had been grown on King's medium B agar (KB) (King et al. 1954) for 24 hours at 25ºC were spread onto fresh Petri plates containing the same medium. After 48 hours at 25ºC, bacterial cells were harvested by washing with sterile deionised water (SDW) and the concentration

spectrophotometrically determined. The concentration was adjusted to 10^2 cfu/ml and a 100-µl aliquot was added to the Eppendorf tubes containing products and nutrient broth. There was an uninoculated control for all product concentrations, and there was an inoculated control in 1x nutrient broth. After 34 hours of growth at 25°C, 100 µl was removed from each Eppendorf tube (except for the biological products) and placed in a cuvette containing 2.5 ml of deionised water. The optical density was measured at 535 nm. The concentration of Psa cells in the presence of different concentrations of biological products was determined by removing 100 µl, diluting in sterile deionised water and placing on King's medium B agar (King et al. 1954) in Petri plates. After 34 hours of incubation at 25°C, Psa colonies were identified by morphology and counted. The efficacy against Psa was calculated as percent reduction in colony numbers compared with number of colonies in the unamended nutrient broth. For the other products, the efficacy against Psa was calculated by logit transforming the OD 535 values, where logit = ln {(p/1-p)} and p = the proportion of the OD 535 values in unamended nutrient broth. Any negative values were adjusted to zero. Logit values were plotted against the logarithmic transformation of product concentration. The response was thus linerarised. The slope of the linear portion of the transformed data was calculated by linear regression. The effective concentration at which growth was inhibited by 50% (EC_{50} value) was calculated from each linear regression equation for Y = 0, and when Y = - 2.77 for EC_{95} values. A constant value was added to non-transformed data to enable 0 and 100% values to be used in the calculations.

A low EC_{50} value indicates an effective product. If the EC_{95} value is also low, then the EC_{50}/EC_{95} ratio will be close to 1. This indicates total kill at a low dosage and this is a good product.

Table 1: Products tested for growth in nutrient broth.

Product name	abbreviation	active ingredient	% or concentration
Blossom Bless®	BB	*Pantoea agglomerans*	3 x 10^{10} cfu/g
Champ® DP	copper	Copper hydroxide	50
Citrolife®	CL	Not disclosed	0.7
Citrox® BC	Citrox	Citrus extracts	28
		Citric acid	
CropBioLife®	CBL	Bitter orange extract	75
		Organic acid blend	
		Ethanol	
		Yucca shidigera	
Zelam® dodine	Dodine	dodine	10
Elicit®	E	DDAL (not disclosed)	51
		SA (not disclosed)	
Flavourjen Zonix®	Zonix	rhamnolipid	8.5
Fulzyme® Plus	FP	*Bacillus subtilis*	1 x 10^{10} spores/g
KeyStrepto®	KS	streptomycin	17
Phoscare®	Phos	Mono-potassium phosphite	53
		di-potassium phosphite	
Plant Nutrient Synergist®	PNS	Citrus extracts	28
Serenade® Max	Serenade	*Bacillus subtilis*	7.3 x 10^{9} cfu/ml
Superzyme®	SZ	*Bacillus subtilis*	2 x 10^{9} spores/g
		Trichoderma konigii,	
		Trichoderma harzianum,	
		Pseudomonas putida	
Teracep®	Teracep	Hydrogen peroxide	50
		Acetic acid	
		Peracetic acid	

KEY RESULTS (all results must be auditable in terms of access to raw data if required)

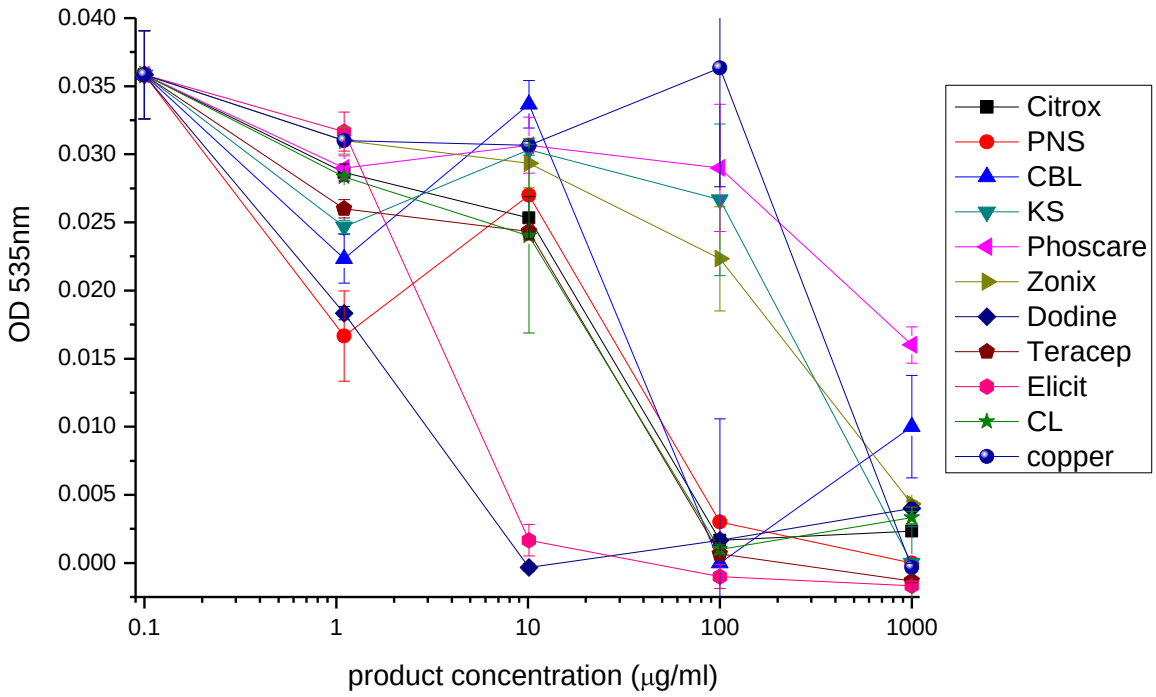

Figure 1. Optical density at 535 nm after 34 hours of Psa growing in the presence of chemical products at 25°C, plotted against concentration. The value of 0.1 is zero plus a constant value so that this value can be included.

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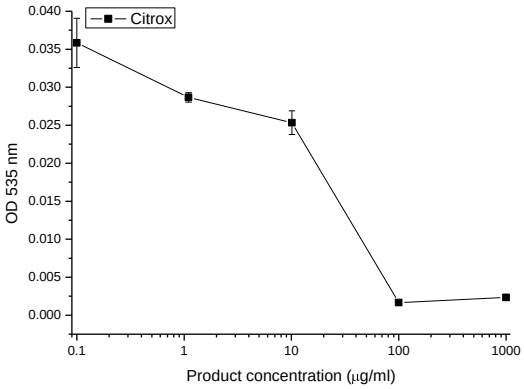

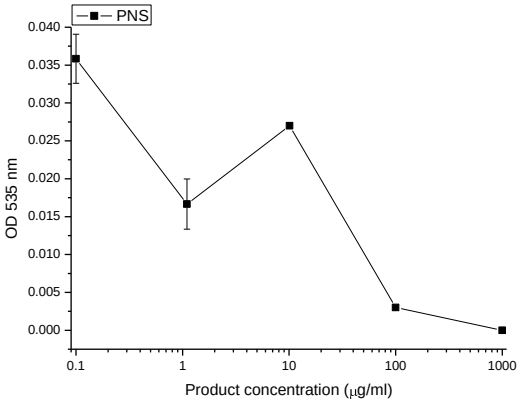

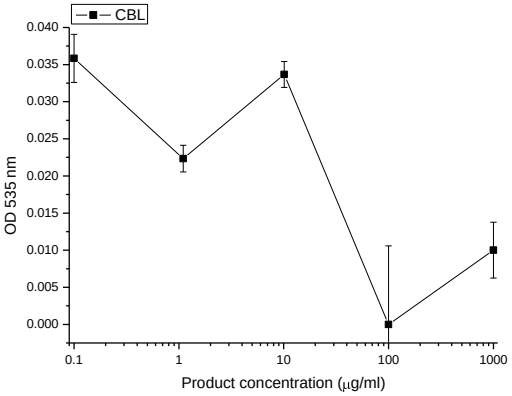

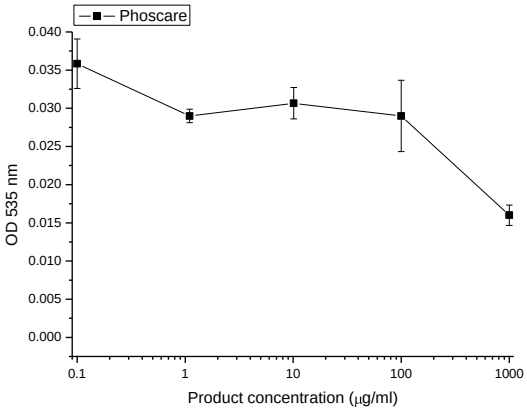

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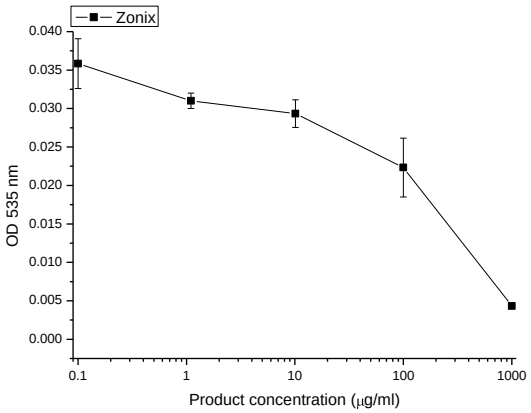

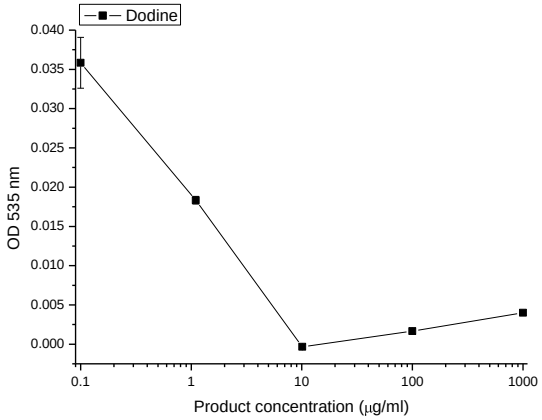

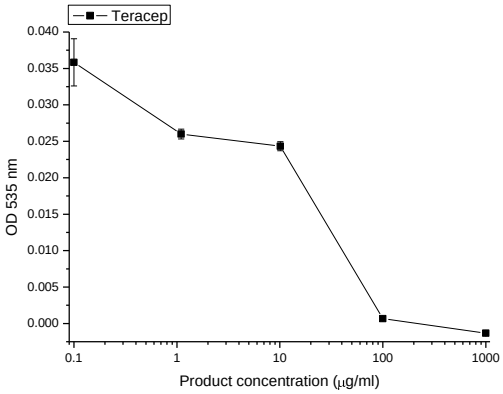

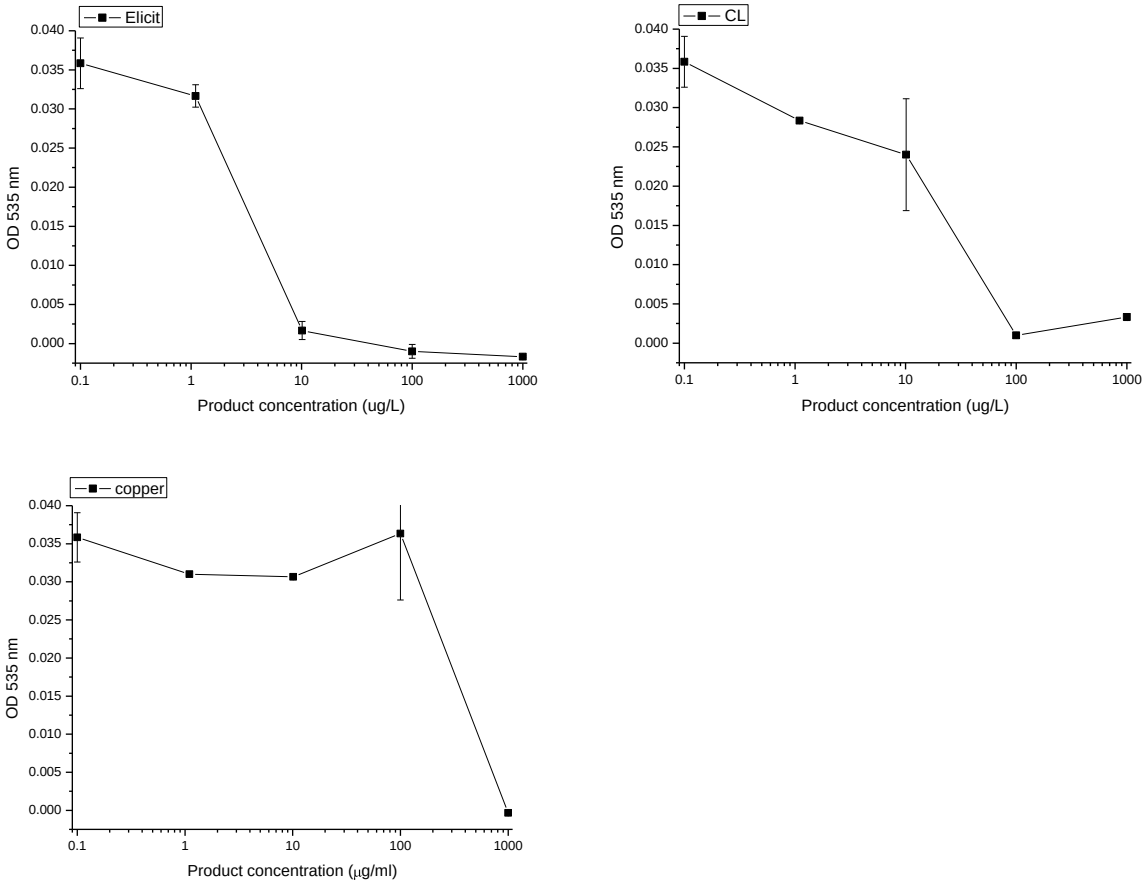

Figure 2. Optical density at 535 nm after 34 hours of Psa growing in the presence of chemical products at 25°C, plotted against concentration for all products except biological control agents. The value of 0.1 is zero plus a constant value so that this value can be included.

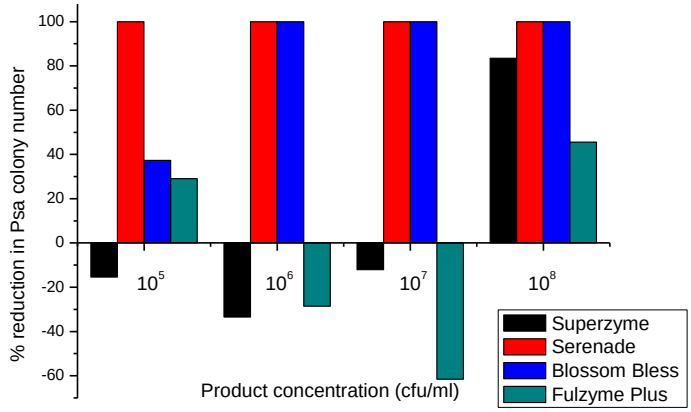

Figure 3. Percent reduction in colony number after 34 hours of Psa growing in the presence of biological control products at 25°C, plotted against product concentration in colony forming units per ml.

Table 2. Effective concentration (EC) in µg/ml at which bacterial cells are reduced by 50% (EC_{50}), 95% (EC_{95}) and a ratio of EC_{50}/EC_{95} as determined by absorbance at 535 nm after 34 hours of growth at 25°C.

	Effective Concentration (EC) in µg/ml		
Abbreviated product name	EC_{50}	EC_{95}	EC_{50}/EC_{95}
Citrox	16.9	87.3	5.2
PNS	21.0	102.7	4.9
CBL	4.8	453.0	95.0
KS	136.2	302.9	2.2
Phoscare	743.8	34715.9	46.7
Zonix	101.5	3949.0	38.9
Dodine	1.0	2.5	2.5
Teracep	2.6	10.8	4.2
Elicit	13.3	70.2	5.3
CL	14.7	66.6	4.5
copper	160.4	334.1	2.1

key to abbreviations – see Table 1.

RECOMMENDATIONS FOR INDUSTRY

All chemical products tested here had some efficacy against Psa. Ideally these tests should be repeated, but because of time constraints, these results will be released. The best products by EC_{50}/EC_{95} ratios were, in order of effectiveness, copper, streptomycin, dodine, Teracep, Citrolife, PNS, Citrox, and Elicit. The product that was most effective at the lowest rate was dodine, followed by Teracep. The biological products were difficult to test using this method, but results showed that Serenade Max and Blossom Bless were the best products, with Serenade Max showing efficacy over a slightly wider range of concentrations than Blossom Bless. In order to validate the results of these tests, field testing is required, as environmental conditions and the presence of plant material can affect the efficacy of these products. However, results of overseas trials have shown that in field conditions both copper and streptomycin reduce disease caused by Psa on kiwifruit (Serizawa et al. 1989). For disinfection of tools, products such as dodine, teracep, Citrolife, PNS, Citrox and Elicit show promise and should be further tested to ensure there is no reduction of efficacy in field conditions.

CONCLUSIONS

Copper and streptomycin were the most effective chemical products by EC_{50}/EC_{95} ratio, and these products have known field efficacy, so can be recommended for field control of Psa. The chemical product that was most effective at the lowest rate was dodine, followed by Teracep, but these products have not been field tested against Psa so cannot be recommended at this stage for that purpose. Of the biological products, Serenade Max was the most effective, but has not been field tested against Psa so cannot yet be recommended for field application.

FUTURE RESEARCH STEPS

Promising products should be tested as tool disinfectants, and in the case of the biological control agents, small inoculated kiwifruit plants could be used to test these materials further in the laboratory or the glasshouse. Field spray trials should also be conducted to test promising products further.

REFERENCES

King EO, Ward MK, Raney DE 1954. Two simple media for the demonstration of pyocyanin and fluorescin Journal of Laboratory and Clinical Medicine 44: 301-307.

Serizawa S, Ichikawa T, Takikawa Y, Tsuyumu S, Goto M 1989. Occurrence of bacterial canker of kiwifruit in Japan: description of symptoms, isolation of the pathogen and screening of bactericides. Annals of the Phytopathological Society of Japan 55(4): 427-436.

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This report has been prepared by The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research), which has its Head Office at 120 Mt Albert Rd, Mt Albert, Auckland.

This report has been approved by:

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