Psa Products Review

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Prepared for Zespri Group Ltd

Roger Gilbertson – Principal, Gilbertson Associates Ltd

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The changes are listed in the table below:

No.	Version date	Section Changed	Change(s) Description
1.	29 March 2013	The final draft edition	n/a
2.	10 April 2013	Final edition	Updated references to testing undertaken. Justification of product testing priority list. Inclusion of PGR's

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1. BACKGROUND

Pseudomonas syringae pv. actinidae (Psa) product testing by Zespri/KVH started soon after Psa was first discovered. Initial testing was relatively ad hoc in an early attempt to find a quick win solution.

Large numbers of products were put forward at a time when a number of innovation strategies were being considered placing a strain on available resources. Significant expectations were placed on finding the Psa control product solution making it vital that testing was done in a methodical and transparent yet efficient way.

The formalised product testing approach including in-vitro and greenhouse testing was introduced early on to provide an ordered and systematic approach to screening different actives. Field trials commenced in November 2011 (approximately a year after Psa was first identified), picking out the more successful products from in-vitro and greenhouse testing to determine their impact in a 'true to life' manner.

Many of the initial product offerings were put forward by the agrichemical industry with formulations that had a range of bacterial impacts. Researchers (incl. Zespri, Plant & Food, and consultants) have also completed investigations and data reviews for other possible chemical, physiochemical and biological options.

The range of options for the front end testing by Zespri/KVH has largely being completed. The objective of this report was to do identify any possibilities that may have been missed.

2. WHAT HAS BEEN TESTED?

2.1 Initial test lists

Initial Psa product test lists focused on potential bactericides already on the market and commercially available (or close to). Consequently these were an array of possible 'winners' from other perennial crops that have a history of bacterial disease issues (e.g. fireblight and pipfruit, blast and stonefruit). The challenge for the kiwifruit industry was that many of the other bacterial diseases are focused on managing the disease at critical times (i.e. reducing the inoculum) while for Psa (on Hort16A in particular) it was more about trying to find something that combated the bacterium directly.

2.2 Grouping of products

Products have been grouped into various categories based on the method each group of chemicals uses to combat Psa. This is extremely helpful as the more successful Psa products may be more about how the chemistry interacts with the vines and not just the active ingredient itself. Ultimately it will be about how these different categories of products then interact to give the best multi-faceted control approach.

The categories have also been extremely important in determining the best test protocol used for each product to ensure its potential efficacy is maximised.

The groupings as described by Zespri/KVH;

Product group	Mode of Action	Possible Use against PSA
Coppers	PROTECTANT. Application of protectant sprays is considered best practice in protecting against Psa-V. When dissolved in water, copper ions (Cu++) are released, bind with proteins in bacteria, and disrupt their function (ie. denature).	Copper Sprays are tried and true bacterial disease protectants sprays used on-orchard. Currently, the most effective Psa-protectant sprays are copper based although their optimal use remains unknown.
Elicitors (SAR products)	ELICITOR. Elicitors are products that induce the plants defence mechanisms allowing them to fight infection. In other crops they have been shown to provide control, similar to that described for biological sprays. Generally, there is a time delay between the spray application and the elicitor effect being activated and the effect is relatively short term requiring additional applications.	Understanding the Psa lifecycle is important in getting the timing correct to provide maximum benefit. Plant defence mechanisms can be quite specific to the pathogen attacking the plant and the plant itself. It is important to gain an appreciation of the mechansims of the plant defence mechanisms in play for the Psa/kiwifruit combination.
Disinfectants/ Sterilants	BACTERICIDAL. Disinfectants/sterilants are substances that are applied to non-living objects to destroy microorganisms that are living on the objects. Disinfectants work by destroying the cell wall of microbes or interfering with the metabolism.	These products will kill Psa easily *in vitro*. However, they often are not persistent and fail to provide long-term protection. They also run the risk of removing all bacteria, creating an environment where harmful bacteria return rapidly.
Biological Control	PROTECTANT/BACTERICIDAL. Biological control agents' can have a dual mode of action. They can provide this in a number of ways including: • occupying the sites the pathogen would normally reside in; • competing against the pathogen for food; • producing anti-bacterial compounds that kill the pathogen.	Biological control may potentially provide a robust means of controlling Psa. The challenge is providing an environment where the control agent can maintain its effectiveness. The screening of Psa specific control agents will be important to deal to the complex mechanisms at work consequently relying on off the shelf solutions will be limiting.
Antibiotics (Streptomycin)	BACTERICIDAL Streptomycin is characterized chemically as an aminoglycosidic antibiotic and is bactericidal in action. All aminoglycoside exert their inhibitory action by blocking protein synthesis in bacteria. Streptomycin is not highly systemic in the vine when applied in a spray formulation so from a testing method it has been treated as a protectant.	There is a trade off with the use of streptomycin. On one hand it has the ability to protect against disease and assist the health of the plant; while on the other hand there is a potential risk of bacteria building resistance. The R&D programme is researching this as the use of streptomycin overseas has proven to restrict and kill the growth of Psa. There will be controlled rules of use associated with best practice, following a better understanding of streptomycin in-field.

2.3 Products showing efficacy

Through time, the list of effective chemicals has grown, however none would be considered a 'winner' in their own right. The approved agrichemicals form part of an overall crop protection strategy with various strengths and weaknesses meaning their strengths are more about working in tandem with other agrichemicals to provide the best overall defensive strategy.

A summary of products showing efficacy on one or all of the kiwifruit varieties can be found on the KVH website http://www.kvh.org.nz/product_testing. Not all have made it in to the Zespri Crop Protection programme for various reasons (including market access implications) or have restricted use patterns.

The more traditional products (coppers, streptomycin) are protectant in nature so are reliant on providing a protective barrier during the high risk timeframes. With only limited knowledge of epidemiology, the industry needed to gain a rapid understanding of when and how to protect the vines. A concerted drive to better understand Psa epidemiology broadened out the net for products that elicit plant defence mechanisms or act in a systemic fashion in the plants.

3. OTHER SECTOR RESPONSES

While bacterial diseases are relatively new to the kiwifruit sector, other sectors have been battling them for a number of years. Unsurprisingly the product mixes and screening undertaken by Zespri/KVH is comparable to other sectors battling with bacterial issues.

Annual crops have greater flexibility in the management of bacterial diseases as they have options of using disease free plants or seeds plus the option to remove the diseased plants at the end of their production cycle and/or introduce crop rotation to break the disease cycle. Although they may have a wider range of methods there is still a major focus on managing/controlling the disease during the production cycle so the product mix is still vitally important.

Other sectors have benchmarked the more traditional (and effective) copper products and antibiotics options against recently developed management options. These management options tend to focus on products from one or a combination of the biological, elicitor and nutritional categories. This approach only adds one or two new options to the mix each time. In comparison, the kiwifruit sector has started from a very limited base knowledge and has needed to reconfirm the traditional product efficacy first.

A recent IR41 project study of bacterial disease in ornamentals tested a wide range of products for the various bacterial pathogens found on ornamentals in North America. The summary list of products is contained in Appendix 3. From this product mix there are some possible additional options to include in a future kiwifruit product testing mix.

When comparing to the other sectors, the mix of products tested for kiwifruit appears to be somewhat weaker in the biological and nutritional spaces in particular. Both are understandable;

- Biological testing is highly complex and host specific so 'off the shelf' solutions are less likely to transfer readily to kiwifruit. Biological testing has recently gained momentum with antimicrobial

[1] Specialty crops project funded by USDA NIFA and headquartered at Rutgers University, New Jersey providing safe & effective pest management solutions for specialty crop growers.

peptides recently developed by Auckland University are being considered for testing by Zespri/KVH (A. Mowat, personal communication, April 2, 2013).

- Nutritional management options tend to be explored once product testing options are exhausted. The impacts of soil nutrition and growing media on Psa are currently being explored as part of a Zespri/KVH project being undertaken by GroPlus (http://www.kvh.org.nz/vdb/document/659).

4. ENVIRONMENTAL SCANNING 2012/2013

Until 2012, product testing largely focused on agrichemical industry presented products that were either commercially available or were in development. In early 2012 an environmental scan was undertaken to find other less commercially available bactericides in an attempt to broaden the list of active ingredients in the mix and chemical groups.

4.1 Products recommended by King & Associates (2012)

A list of likely and potential compounds was provided to Zespri/KVH by consultants, King & Associates, in early 2012. This list has been reviewed as part of this report to identify products that have been tested already versus those that haven't. The list is summarised in Appendix 1 and forms part of the recommended Psa products test list shown in section 6.

4.2 Plant & Food screen (2013)

Plant & Food undertook a subsequent product screening in 2013. Using a profiling technique called Phenotype Microarray's; P&F screened a total 240 possible products. Out of this total 61 products showed some biostatic/biocidal activity.

This list however contains a number of products that may not be legally or ethically able to be used as a plant bactericide. The summary list in Appendix 2 identifies those that would be acceptable for further testing and will form part of the recommended Psa products test list shown in Section 6.

5. REVIEW OF ALTERNATIVE APPROACHES

There is evolving knowledge in battling bacterial disease which investigates new chemistries, biological control options and plant defence responses.

5.1 Bacteriophages

Phages are viruses that infect bacteria. Mixtures of phages can be produced that are specific to bacterial populations. One of the major challenges with this technology is to maintain a viable phage population on the foliage for an adequate length of time. Agriphage has demonstrated success in control of bacterial leaf spot in tomatoes (Obradovic *et al* 2004) although Ivors (2006) could not demonstrate the same effect when comparing it to other products in trials on tomatoes.

Testing to date by Zespri/KVH of phages from Omnilytics (in greenhouse and in the field) has not shown them to be effective in either a field trial capacity (www.kvh.org.nz/vdb/document/91136) or in a greenhouse situation (www.kvh.org.nz/vdb/document/821). Bacteriophage carrier systems (i.e. bacteria that carry phages into plants) to improve the performance of phages have been identified by the University of Otago and will be evaluated in by Zespri/KVH (http://www.kvh.org.nz/vdb/document/557).

5.2 Bacteriocins

Bacteriocins are substances produced by plant pathogenic bacteria that antagonise other closely related bacteria. Avirulent (non-disease producing) variant of the bacterial pathogens that produce the desired bacteriocins would have to be developed along with a way to formulate for crop use.

Recently, Landcare Research has identified bacteriocins from *Pseudomonas spp.* (A. Mowat, personal communication, April 2, 2013). The author assumes that Landcare Research and Auckland University will continue the screening of bacteriocins and other biological control agents.

5.3 Alternative biocontrol agents

5.3.1 Trichoderma

Both secondary metabolites derived from Trichoderma (anthraquinones) and plant systemic response to soil applied *Trichoderma asperellum* show potential for further investigation. The BioProtection group at Lincoln University has recently identified Trichoderma with potential to control Psa (http://www.kvh.org.nz/vdb/document/91139). These are currently being evaluated by Plant and Food Research and Zespri/KVH.

5.3.2 Anti-microbial peptides/lipopeptides

Peptides derived from kininogen, with end-tagging resulted in enhanced bactericidal effect against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* (Pasupuleti et al 2009). The higher bactericidal potency of the tagged peptides correlated to a higher degree of binding to bacteria, and resulting bacterial wall rupture.

As outlined previously, the University of Auckland has developed peptides for further evaluation.

5.4 SAR products/Plant defence mechanisms

5.4.1 Systemic acquired resistance pathways

Understanding and targeting the plant defence mechanism has provided a range of positive product options for Psa management (e.g. Actigard, Spotless). The majority of the work to date has focused on the Salicyclic Acid (SA) stress signalling mechanism in kiwifruit.

Phytohormones play central roles in both abiotic and biotic stress signalling. Salicylic Acid (SA), Jasmonic Acid (JA) and Ethylene (ET) have central roles in biotic stress signalling. SA is involved in resistance to biotrophic pathogens (which includes Pseudomonas) and JA–ET is involved in responses to necrotrophic pathogens.

Systemic acquired resistance (SAR) is a pathogen induced defence mechanism effective against a wide range of pathogens. SA plays an important role in this plant defence response to pathogen attack. Studies suggest that one of SA's mechanisms of action is the inhibition of catalase, resulting in elevated levels of hydrogen peroxide, which activate defence-related genes. SA has also been shown to inhibit ascorbate peroxidase, a key enzyme for scavenging Hydrogen peroxide.

Psa product testing to date has included a focus on elicitors of this SAR pathway potentially without fully exploring how they impact the SAR pathway in kiwifruit. There may be benefits in deconstructing the SAR pathway to consider other products that could be instrumental in activating defence related genes.

Recent advances in the science communities understanding of plant defence signalling have revealed that plants employ a network of signal transduction pathways, some of which are independent of salicylic acid. Evidence is emerging that JA and ET play key roles in these salicylic acid-independent pathways. Cross-talk between the salicylic acid-dependent and the salicylic acid-independent pathways provides great regulatory potential for activating multiple resistance mechanisms in varying combinations.

Vernooij *et al* (1995) identified a synthetic chemical 2, 6-dichloroisonicotinic acid (INA) acts via the SAR pathway but doesn't induce SA accumulation. They found instead that INA apparently activates a component of the SAR signalling pathway downstream of SA accumulation. This becomes important for those plants that cannot accumulate SA.

5.4.2 Plant Growth regulator impact on pathogens

Kennelly et al (2007) cited a number of studies that showed the growth regulator prohexadionecalcium (trade name Regalis in New Zealand) inhibited fire blight of apple, through a reduction of succulent shoot growth, thereby limiting the availability of highly disease-susceptible shoots. Spinelli *et al* 2005 suggested that prohexadionecalcium stimulates host resistance by triggering production of the antimicrobial compound luteoforol. Regalis has not shown the same degree of success during Psa product testing but other PGR's are demonstrating a somewhat better effectiveness (e.g. forchlorfenuron – CPPU). Studies in Italy have shown a positive impact of 3ml/litre forchlorfenuron on reducing the incidence of Psa on kiwifruit vines. In contrast treatment with 3,5,6 TPA had no or negative impact on the incidence of Psa. The specific resistance mechanism that forchlorfenuron triggers is not clearly understood at this point but does warrant further investigation.

5.5 Multi product testing combinations

To date there have been very few multi product tests undertaken. This is understandable as the efficacy of a single product needs to be understood to ensure it has potential in its own right. A number of studies have indicated the possible synergistic effects of a multi-product approach.

5.5.1 Mixing Biological control agents (microbial strains)

Demonstrating the effectiveness of biological control agents can be challenging. The concentrations of the pathogen required to provide clear symptoms on the vines may consequently be too high for the biological to demonstrate effectiveness. In part this may explain why biological products do not feature strongly in the effective Psa products mix and why Zespri/KVH is exploring other bioassays to demonstrate biological efficacy.

Samiyappan *et al* (2007) cites a number of studies that demonstrate an improved performance in disease reduction when mixtures of microbial strains were applied as seeds treatments or as sprays to a range of crops (e.g. cucumbers, tomatoes, pears).

In another study, bacterial blight of anthurium (caused by *Xanthomonas campestris*) was suppressed by several bacterial strains indigenous to leaves of various anthurium cultivars. The individual strains in this community had no effect on the pathogen, but the mixture was inhibitory to *X. campestris* pv. *dieffenbachiae* in guttation fluids (Fukui *et al* 1999).

Although the context of these studies is different to Psa product testing there is some indication that a mix of strains may have some collaborative benefit. A further study on mixes of biocontrol agents in kiwifruit antagonistic to Psa is recommended.

5.5.2 Elicitor mixes

In a similar vein, there is an opportunity to study the interaction between Psa product groups with elicitors.

Chitosan on its own provided a limited positive response during Psa testing. However, a combination of Plant Growth Promoting Rhizobacteria (PGPR) strains, *Bacillus subtilis* (GBO3) and IN937a (*B. amyloliquefaciens*) with the carrier chitosan lead to the protection against CMV in tomato (Murphy *et al* 2003). This raises a question, does the chitosan enhance the performance of the microbial components or does it act independently but collaboratively with the microbial mix?

As discussed previously, the different point of focus of INA on the SAR pathway from salicylic acid may result in an improved overall performance when the two chemicals are combined.

6 RECOMMENDED LIST FOR PSA TESTING

There has been a range of reviews and identification of new bactericidal materials from various sources. The following table brings together the different sources of information into a recommended list for the next stage of Zespri/KVH Psa testing. The list has been split into a priority list (Table 1) and a Secondary testing list (Table 2). Other potential products/compounds with a lower priority for testing are listed in Appendix 1, 2 and 3 although it is anticipated these products either require further fundamental research to be conducted first or the demonstrated benefit are somewhat less then the products shown in Tables 1 and 2.

It is possible that some of these products/compounds identified in this report have already been tested and that the author of this report was not aware of this.

Table 1: Priority testing list

The following list represents the actives that show the most promise with demonstrated direct activity against bacteria that have at this stage not been tested for efficacy against Psa.

Active ingredient	Products	Justification for inclusion	Source of information
2,6-Dichloroisonicotinic acid	None found	Triggering SAR pathway without reliance on SA. May have benefit for varieties that cannot accumulate SA or in combination with elicitors that trigger SA.	Vernooij et al 1995
Copper salts	Camelot	Copper salts of fatty and rosin acids. A different formulation of copper from that previously tested. IR4 project showed good control on *Xanthomonas sp* on geraniums but not tested on *Pseudomonas sp*.	Appendix 3
Chlorophenol	Applied 3-78	Found to be one of the most bactericidal against bacterial canker of tomato out of 27 products tested and least phyto toxic.	Appendix 1
Flumequin	Firestop; Fructil	Useful for spraying against fire blight and bacterial dieback and is active against numerous gram-negative bacteria. As effective as Bordeaux in control of fireblight/bacterial dieback but less effective than antibiotics.	Brisset et al 1991
Octylaminoethyl glycine hydrochloride	None found	Ranked one of the highest of 13 bactericides against the causal organism of rice bacterial foot rot. Utilises a resin containing acrylonitrile & styrene &/or diene as units. Limited knowledge of product.	Appendix 1
Tecloftalam	Tecloftalam (BSI, ISO-E); Técloftalame (ISO-	Used to control leaf blight caused by the bacterium *Xanthomonas campestris pv oryae* in paddy fields.	Appendix 1

	F).	Limited knowledge of product.	
Dihydroxy-octadecanoic acid	Patented technology	DOD showed antibacterial activity against food-borne pathogens and plant pathogenic bacteria. Physiological activity tests revealed DOD inhibited the growth of *Pseudomonas syringae pv. sesami, Pseudomonas syringae pv. actinidae, Pseudomonas syringae pv. syringae.* Limited knowledge of product	Appendix 1

Table 2: Secondary investigation list

The following are the recommended actives or actives of interest from Appendix 1 (King and Associates) and Appendix 2 (P&F screening - demonstrating efficacy). There is limited information on the efficacy as a bactericide but sufficient related information to suggest further investigation is warranted.

Active ingredient	Products	Justification	Source of information
Acriflavine		Antiseptic used against external parasitic infections in freshwater and marine tropical ornamental fishes. No clear reference as a plant antimicrobial.	Appendix 2
Benzethonium Chloride		QAC compound similar in mode of action to Benzalkonium Chloride already tested in various formulations with some success.	Appendix 2
Cetylpyridinium chloride		A QAC found in cough lozenges and syrups; emulsifier; laboratory reagent.	Appendix 2
Dequalinium chloride		Antibacterial and antifungal agent (QAC) active against many Gram-positive and Gram-negative bacteria. Now licensed only as a topical medication.	Appendix 2
Dioctyldiethylenetriamine acetate	Xinjunan	An effective bactericide and virucide widely used on many crops including vegetables and fruits in China. No efficacy data seen.	Appendix 1
Ethylicin (none found)	Ethylicin	One of 3 fungicides (total screen of 14) found effective in control of canker disease in Chinese hickory caused by *Macrophoma caryae.*	Appendix 1
Fenaminosulf	Lesan, Dexon, Bayer 22555, Bayer 5072, diazoben	Good antibacterial effect against Mango bacterial black spot disease caused by *Xanthomonas campestris pv. mangiferae-indicae*	Appendix 1
Gallic acid		Water soluble phenolic acid present in grapes and in the leaves of many plants. Appears to have antioxidant, anticarcinogenic and antiangiogenic activity *in vitro*.	Appendix 2
Glycine hydroxamate		Inhibits Glycine decarboxylase in tobacco. Unclear of the implications of this product in context of Psa.	Appendix 2
Hinokitiol	Hinokitiol (skin care products)	A natural biocide to control postharvest decay pathogens. The compound inhibited *in vitro* spore germination and mycelial growth of *Botrytis cinerea* and	Appendix 1

		Alternaria alternata	
Oleandomycin, phosphate salt		Oleandomycin suppresses the growth of Gram-positive bacteria, primarily staphylococci, streptococci, and pneumococci.	Appendix 2
Phenazine oxide (also called azophenylene, dibenzo-p-diazine, dibenzopyrazine, acridizine)	Phenazine	Phenazine compounds are found in nature and are produced by bacteria such as *Pseudomonas spp*. A low toxic fungicide. Protective effect in the control of rice bacterial blight, and hybrid rice blast	Appendix 1
Polyhexamethylene guanidine	Devazid	Used as a sanitizer in a wide range of applications. It is used to preserve wet wipes; to control odour in textiles; to prevent microbial contamination in sterile dressings; and as a disinfectant in a range of environments including recreational water treatment.	Appendix 1
Proflavine		Acridine derivative. Slow-acting disinfectant with bacteriostatic action against many Gram-positive bacteria but less effective against Gram-negative organisms.	Appendix 2
Sodium Azide		Used for air bag inflation; preservative in diagnostic medicines; intermediate in explosives manufacture; in weed and fruit rot control. Also used as a pre-plant replacement to Methyl Bromide.	Appendix 2
Thiodiazole-copper	Thiodiazole-copper	Claimed to be organic, have systemic properties. No scientific studies.	Appendix 1
Famoxadone + Cymoxanil	Tanos 50DF, SP2015	In IR4 study showed limited efficacy against *Pseudomonas sp.* on its own but highly effective in combination with Kocide.	Appendix 3

7 ADDITIONAL RECOMMENDED APPROACHES FOR PSA PRODUCT TESTING

To date the approach has included environmental scanning for potential products and testing these one by one. This approach has provided a substantial list of possibilities that have already been tested or are recommended to be tested. Although the list is not exhausted it is noticeable that the likelihood of unearthing a random winner is becoming less likely. To ensure the value of product testing continues a number of additional approaches to product testing are recommended;

<u>Copper and Streptomycin resistance management testing</u>
It is understood resistance management is part of the Zespri/KVH R&D programme strategy to ensure the maximum value from copper and antibiotic products in particular. From a product testing option perspective there are various additional products inclusions that could be applied in a field trial perspective.

- Control of copper resistance can be improved by increasing the quantity of available copper in spray solutions. One way this can be accomplished is by tank-mixing EBDC fungicides (Cornell University

online fact sheet - Managing Bacterial Diseases of Tomato in the Field). Please note; there are on-going concern on the suitability of EBDC fungicides.

- One of the major antibiotic resistance mechanisms in a range of gram negative bacteria is the Resistance Nodulation Division (RND) efflux pump. Work has been done to investigate to identify compounds that inhibit the RND-type efflux pumps. (Kamicker et al 2008)

Reverse engineer the SAR pathway

As discussed previously in this report the primary focus in testing of elicitors has been the role of salicylic acid (SA) and how to induce the plant to trigger this mechanism or applying SA exogenously. A range of work suggests that in the case of kiwifruit and Psa there is benefit in reverse engineering the SAR pathway to better understand the mechanics of various phytochemicals in the SAR pathway and subsequently identify other compounds that may trigger the plant defence mechanism (e.g. 2, 6-Dichloroisonicotinic acid - INA)

Multi-product testing programme

The synergistic benefits of mixing various compounds have been demonstrated in other disease management studies. Mixing within groups (e.g. microbial strains) or mixes between groups (e.g. elicitors and biological) have improved the overall control of the disease then when applied individually.

Programme approach to product testing

The recommended Psa management programme has largely evolved by combining individual products demonstrating efficacy which are Zespri approved. There is an opportunity to take this a step further and compare the efficacy of various programmes on the long term control on the vines.

Trials by Roberts et al (2008) showed a 6 applications of Actigard alternated with 5 applications of copper mancozeb provided either the equivalent or better (38% less disease) than a standard copper-mancozeb programme.

A programme approach has been identified by Zespri/KVH as important to test. To this end, a spray programme project is being funded by Zespri/KVH. In this, combinations of products applied during a season are being evaluated on a small number of orchards.

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Appendix 1: King and Associates review list

A1.1 TESTED (in either/or *In-vitro* or *in-vivo*)

Product	Active ingredient
Beltanol; Chinosol; Probelte	8 Oxyquinoline, Hydroxyquinoline sulfate
Bronotak, Bactinash, BIOBAN BP-M Antimicrobial	Bronopol
Syllit, Melprex, Zelam Dodine(NZ) 2439-10-3	Dodine
Oryzemate®	Probenazole
Pyrithione (zinc or sodium) - Dandruff shampoos	2-Mercaptopyridine N-oxide sodium salt hydrate, 98%
Sovran®; Stroby WG, Kresoxy WG, Beem WG, Candit	Kresoxim methyl
Apron XL; Ridomil; Speartek	Metalaxyl
Drogen, Bismerthiazol 20% WP	Bismerthiazol (in-vitro only)
Saisentong	Saisentong (in-vitro only)

A1.2 NOT TESTED

Product	Active ingredient
Applied 3-78	Chlorophenol
None found	Octylaminoethyl glycine hydrochloride
Tecloftalam (BSI, ISO-E); Técloftalame (ISO-F).	Tecloftalam
Lesan, Dexon, Bayer 22555, Bayer 5072, diazoben	Fenaminosulf
Firestop; Fructil	Flumequin
Hinokitiol (skin care products)	Hinokitiol
Phenazine	Phenazine oxide (also called azophenylene, dibenzo-p-diazine, dibenzopyrazine, acridizine)
Thiodiazole-copper	Thiodiazole-copper
Ethylicin	Ethylicin (none found)
Xinjunan	Dioctyldiethylenetriamine acetate
Devazid	Polyhexamethylene guanidine

Additional products/actives list

No product name listed	Amicarthiazol
No product name listed	Cellocidin
No product name listed	Chloramphenicol
Balear, Barrack, Barrachlor, Blizzard, Bravo weatherstik, Cavalry, Chlorothalonil.	Chlorothalonil
Gallex	Cresol
none found	Emodin
No product name listed	Hexachlorophene
No product name listed	Hydrargaphen
ISOLAN® GPS	Isolan
No product name listed	Nitrapyrin
Recoil, Ripost,Sandofan	Oxadixyl
Saijunmao W.P	Saijunmao
No product name listed	Thiomersal

Multiple	Thiophanate methyl
"Calixin 86" (BASF)	Tridemorph
No product name listed	Zinc thiazole

Alternatives

Broad category	Specific compound	Comments
Plant antimicrobials (Anthraquinones)	Chrysophanol, physcion,rhein, berbine, methyl glyoxal (manuka honey), allicin (garlic)	Early stages of understanding and maximising efficacy of these products. Allicin previously tested.
MDR (multi-drug resistant) pump inhibitors	Compounds that disable of MDR pump (decrease permeability)	Plant antimicrobials might be developed into effective, broad-spectrum antibiotics in combination with inhibitors of MDRs
Anti-microbial peptides Peptides triggering Systemic resistance	Lipopeptides	A new family of synthetic, membrane-active, ultrashort lipopeptides produced expression of defense-related genes in cucumber and Arabidopsis seedlings. Peptides derived from kininogen, and truncations thereof, with end-tagging resulted in enhanced bactericidal effect against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*.
Trichoderma	Secondary metabolites derived from trichoderma-anthraquinones. *Trichoderma asperellum*	Anti-microbial application of the secondary metabolites and systemic resistance of soil applied *T.asperellum* worth further investigation.
Fatty acid	Dihydroxy-octadecanoic acid (patented technology)	The agent exhibited high antibacterial activity against various microorganisms including both Gram positive and Gram negative bacteria
Phytohormone	Nitric oxide (NO)	Bacteria establish intimate associations with plants and therefore NO metabolism by one organism can influence the physiology of the other.
Systemic resistance	Flagellin perception	

Key

Highest priority testing

Worth investigating further (higher priority) (lower priority)

Previously or currently tested

Appendix 2: Plant & Food screening list

Chemicals selected with a reading of <0.05 (hence plated) for 4 isolates, with no growth on droplet for at least 3 isolates for any of the lowest 3 concentrations plated	
Nafcillin	Sodium orthovanadate
Enoxacin	Oleandomycin, phosphate salt
Erythromycin	Sodium Azide
Benzethonium Chloride	Rifamycin SV
Dequalinium chloride	Sodium periodate
Rolitetracycline	Semicarbazide hydrochloride
Acriflavine	Benserazide
Cadmium chloride	Proflavine
Sodium Nitrite	Oxytetracycline
Chemicals selected with a reading of <0.05 (hence plated) for 4 isolates with no growth at the highest concentration for all isolates	
Chlortetracycline	Chlorpromazine
Colistin	Pipemidic Acid
Enoxacin	Lidocaine
Penimepicycline	Gallic acid
Tylosin	trans-Cinnamic acid
Sodium Cyanate	Phenethicilllin
EDTA	DL-Propanolol
Oleandomycin	Atropine
Cetylpyridinium chloride	Dodine (n-Dodecylguanidine)
Glycine hydroxamate	4-Hydroxycoumarin
Lowest mean readings at 48 hours. Some isolates grew from droplet	
Oleandomycin	Sulfadiazine
Compound 48/80	Thallium (I) acetate
Methyl viologen (Paraquat)	Sodium azide
Sodium m-periodate	5-Fluoroorotic acid
Benzethonium chloride	Sodium metavanadate
Enoxacin	Sulfanilamide
Orphenadrine	Azlocillin
Oxytetracycline	Penimepicycline
Phenethicillin	Sulfathiazole
Oxacillin	Pipemidic Acid
Tetracycline	D,L-Propranolol
Amitriptyline	Sodium Selenite

Key

Not recommended for testing by P&F
Not recommended for testing (Medical or Veterinary use, antibiotic)
Worth investigating further (higher priority) (lower priority)
Previously tested

Appendix 3: IR-4 Ornamental Horticulture Program Bacterial Disease Efficacy

Efficacy tested against:

Erwinia amylovora	***Pseudomonas* spp.**
Erwinia chrysanthemi	***Pseudomonas syringae***
***Erwinia* sp.**	***Xanthomonas campestris***
Pseudomonas chicorii	***Xanthomonas* spp.**
Pseudomonas marginalis	

Authors: Ely Vea and Cristi Palmer
Date: May 23, 2012

Products used in testing:

Acibenzolar	Kasumin (Kasugamycin)
Actinovate NI108 (*Streptomyces lydicus*)	KleenGrow (Didecyl dimethyl ammonium chloride)
Agri-Mycin (Streptomycin)	Kocide (Copper hydroxide)
Alexin (Various nutrients)	K-Phite (Phophorus acid salts)
Aliette WDG (Fosetyl-Al)	Milstop (Potassium bicarbonate)
ASAP (silver)	NAI-4201 (Tiadanil)
BioPhos (Dipotassium phosphonate + Dipotassium phosphate)	Omega-Grow Plus (Fish oil)
BloomTime (*Pantoea agglomerans*)	Penncozeb (Mancozeb)
Camelot (Copper salts)	Phyton 27 (Copper sulfate pentahydrate)
Cease (*Bacillus subtilis* strain QST 713)	Protect (Mancozeb)
CG100 (CG100)	Regalia SC (MOI-106), Milsana (*Reynoutria sachalinensis extract*)
Champ 2F (Copper hydroxide)	ReZist (Chelated Copper+Mn+Zn)
Citrex (Citrus extracts)	SP2015 (Famoxadone + Cymoxanil)
Companion (*Bacillus subtilis* GB03)	Starner (Oxolinic acid)
CuPRO (Copper hydroxide)	Taegro (*Bacillus subtilis var. amyloliquefaciens* strain FZB24)
Cuprofix (Copper sulfate)	Tanos (Famoxadone + Cymoxanil)
Cuprofix MZ (Copper sulfate+mancozeb)	Tricon (Sodium tetraborahydrate decahydrate)
Dithane 75WP (Mancozeb)	Vital (Potassium phosphite)
Junction DF (Mancozeb+Copper hydroxide)	Vitalonil (Potassium phosphate+Chlorothalonil)

Key

To be considered
Highest priority testing
Worth investigating further (higher priority) (lower priority)
Previously tested