Copper Sprays on Kiwifruit: Bactericidal Effectiveness, Bio-availability & Phytotoxic Effects – Literature Review

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1. Introduction

Copper spraying has become a significant component of the kiwifruit industries spray programme for providing protection against infection by Psa-V. The industry has made significant progress in its understanding of copper use over the 3 years since Psa-V was identified in New Zealand, through field trials and grower experience. However, there are still many questions around the optimising of efficacy and avoiding phytotoxicity that warrant developing a better understanding of how copper works once applied to plants.

This literature review will cover the following topics:

- Brief history of Psa and outbreak in New Zealand
- Mechanisms used to control outbreak of Psa in New Zealand and other countries
- Literature on Cu-based agrochemicals in controlling Psa, and other diseases in fruit crops
- Environmental and plant factors affecting effective control of Psa and other bacteria/fungi by means of Cu-based agrochemicals
- Copper resistance
- Phytotoxic effect of Cu in kiwifruit vines
- Cumulative phytotoxic effect on Cu in soil
- Previous incidence of Cu resistant Psa strains or other bacteria/fungal strains
- Methodology to quantify effective Cu^{2+} ions concentration at both laboratory and field levels (total/bio-available)
- Understand copper chemistry e.g. speciation, complexing and subsequent efficacy
- Methodology to quantify bacteria numbers at both laboratory and field levels
- Research gap and recommendations

2. *Pseudomonas syringae* pv. *actinidiae* – Pathogen history

Pseudomonas syringae is the name of a bacterial pathogen originally isolated from lilac, but subsequently found on a wide range of host plants, each having a host-specific strain (Dye et al., 1980; Young et al., 1978). Spiers (2010) reported that the behaviour of *P. syringae* on poplars in Palmerston North during the 1985-1986 growing season is relevant to the occurrence of *Pseudomonas syringae* pv. *actinidiae* (Psa) in kiwifruit (*Actinidia deliciosa and A. chinensis*) as it will give an insight as to how Psa will perform in NZ. Psa is the current cause of financial losses in many countries around the world, due to its detrimental effects upon kiwifruit vineyards. Psa was first identified and isolated in Japan in 1984 (Takikawa et al., 1989). It was then found in both Italy and South Korea (Koh et al., 1994; Scortichini, 1994). However, the disease did not cause any significant plant losses over a period of 20 years in Italy, but in Japan and South Korea it damaged plantings during this same period of time. In 2008, with the outbreak of sudden and rapid epidemics, Psa was found to be a major threat to kiwifruit orchards in the main regions of Italy (Balestra et al., 2011; Ferrante et al., 2012; Marcelletti & Scortichini, 2011). In 2010 Psa was first reported in New Zealand, and the following year the bacterium caused severe damage to both the green and gold kiwifruit industries (Everett et al., 2011). The pathogen has continued to be discovered all around the world with recent recordings in Portugal (Renzi et al., 2012b), France (Vanneste et al., 2011c), Spain (Abelleira et al., 2011), Switzerland (EPPO., 2011b), Turkey (Bastas & Karakaya, 2011) and Chile (EPPO., 2011a) for the first time. It has also been reported in recent work in South Korea (Koh et al., 2010).

3. Psa in New Zealand and its biovar distribution

The New Zealand kiwifruit industry comprises approximately 2700 orchards from Kerikeri in the north of the North Island to Nelson at the top of the South Island. Eighty percent of the industry's productive orchards are currently located in the Bay of Plenty (BOP), the remaining 20% are located amongst 10 other regional growing centres (Stokes, 2013). Immediately after the discovery of Psa, the Ministry of Agriculture and Forestry (MAF), now the Ministry of Primary Industries (MPI), initiated a bio-security response, and ongoing research is being conducted by various research organizations to develop an efficient kiwifruit management plan for a Psa positive environment. This includes effective control of Psa without compromising the quality of kiwifruit orchards, potentially growing Psa-resistant kiwifruit varieties and understanding how climate factors affect vine susceptibility (Vanneste, 2012).

The results of many recent studies conducted on Psa genomes have indicated that this pathogen is able to rapidly alter its composition by acquiring and/or losing mobile genetic elements and virulent factors and is therefore able to readily adapt to new hosts and environments (Marcelletti et al., 2011). Chapman et al. (2012) conducted a study to characterize Psa populations globally by an in-depth genetic analysis with a representative set of 40 isolates from New Zealand, Italy, Japan, South Korea, Australia and Chile. Through application of multi-locus sequence analysis (MLSA), they classified four distinct Psa groups worldwide and have identified them as Psa1, Psa2, Psa3 and Psa4. Their origins and distinctive histories are given in Table 1. These groups are consistent with the classification proposed by Vanneste et al. (2013).

Table 1 The origin of *Pseudomonas syringae* pv. *actinidiae* biovars (Chapman et al., 2012)

MLSA groups	**Origins**	**Remarks**
Psa1	Japan and Italy	Population found during 1992 outbreak
Psa2	Present in South Korea	
Psa3	From recent outbreak in Italy, the Te Puke region of New Zealand, Chile and China	Related to progression from primary leaf spot symptoms. More advanced secondary symptoms including the production of exudates, and cane and shoot diebacks, in NZ Kiwifruit Orchards (Everett et al., 2011).
Psa4	Currently present in New Zealand and Australia	Genetically distinct from Psa1, Psa2 and Psa3. Much more wide spread in NZ (Chapman. et al., 2011). Has never been observed to progress beyond leaf spot symptoms

A. Disease symptoms

The production of rusty red exudate from infected tissues in early spring is the most visible symptom of Psa (Scortichini, 1994; Scortichini et al., 2012). The presence of the pathogen in the tissues of kiwifruit vines can also be identified by the reddish-brown discolouration under the bark, which is observed even before the production of exudates. The appearance of white exudates is assumed to be caused by the intensely multiplied bacteria that have been pushed out of the infected tissues (Vanneste et al., 2011b). However, this is harder to detect and is not always related to the other symptoms present. Another visible symptom of the disease during spring is the wilting or death of the buds. Buds that are present on infected canes may develop but they will then rapidly wilt and die. Later in the year infected shoots will also wilt, along with leaves which first cup and then dry out. Younger leaves are more susceptible to the disease and they initially express symptoms in the form of small angular necrotic spots surrounded by a yellow halo. The wilting of the plant may be attributed to the vascular system being clogged up with the bacteria (Scortichini et al., 2012).

B. Source of Psa introduction in New Zealand: Pollen?

Pollen and flower material imported from China infected by the bacteria Psa is thought to be the vector by which the pathogen was introduced into New Zealand. Recently published evidence has shown the ability of Psa to survive in *Actinidia* pollen (Stefani & Giovanardi, 2011; Vanneste et al., 2011a; Holmes et al., 2013). However, it is yet to be identified exactly how the infected pollen contributes to the spread of the pathogen. Further studies also showed that the Psa had the ability to survive for up to 45 days in detached organs, such as leaf litter and twigs (Marcelletti & Scortichini, 2011; Scortichini et al., 2012).

C. Climatic Factors favourable for Psa infection in kiwifruit vines

In spring and early summer, the infection develops in expanding shoots and leaves. Small cankers develop on extending vines, and leaves develop angular leaf spots. In the next winter and early spring, extending cankers form on trunks and branches (Serizawa et al., 1994). Psa is most invasive at relatively low temperatures (10-20 °C; optimum 15±3 °C), being almost completely inhibited above 25 °C (Serizawa & Ichikawa, 1993a, 1993b). Serizawa and Ichikawa, 1993c, demonstrated that the Psa populations in kiwifruit vines decreased progressively from late spring to summer when temperatures rose from 18 to 25°C. Serizawa and Ichikawa (1993d) reported that resistance of kiwifruit vines to Psa increased with temperature, such as when average temperatures were above 20°C for at least 10 days; the degree of infection was drastically reduced under these conditions.

In contrast, recently conducted research into the outbreak of Psa in Italy has produced further knowledge about the lifecycle of the pathogen:

(i) the pathogen is able to survive at high temperatures, up to and above 25°C;

(ii) during summer, it is extremely difficult to isolate the bacterium even from the foliar spots which occurred at the beginning of spring;

(iii) Psa is unable to survive for the duration on an entire vegetative season outside of kiwifruit organs;

(iv) Psa can reach the roots and overwinter (Renzi et al., 2012a; Rossetti & Balestra, 2008; Stefani & Giovanardi, 2011).

High relative humidity or rain reduces the latent period between infection and symptom development (Serizawa & Ichikawa, 1993a). Serizawa et al., (1989) reported that the outbreak of the disease increased rapidly after heavy rainfall with strong winds. Ferrante et al. (2012) observed that a clear-cut relationship was present between the occurrence of frost events during winter and the outbreaks of bacterial canker in the following growing season. Based on these observations and their Psa research activities in Italy and New Zealand kiwifruit vines, Vanneste et al., (2011b) summarised a hypothesised life cycle as follows; (Figure 1).

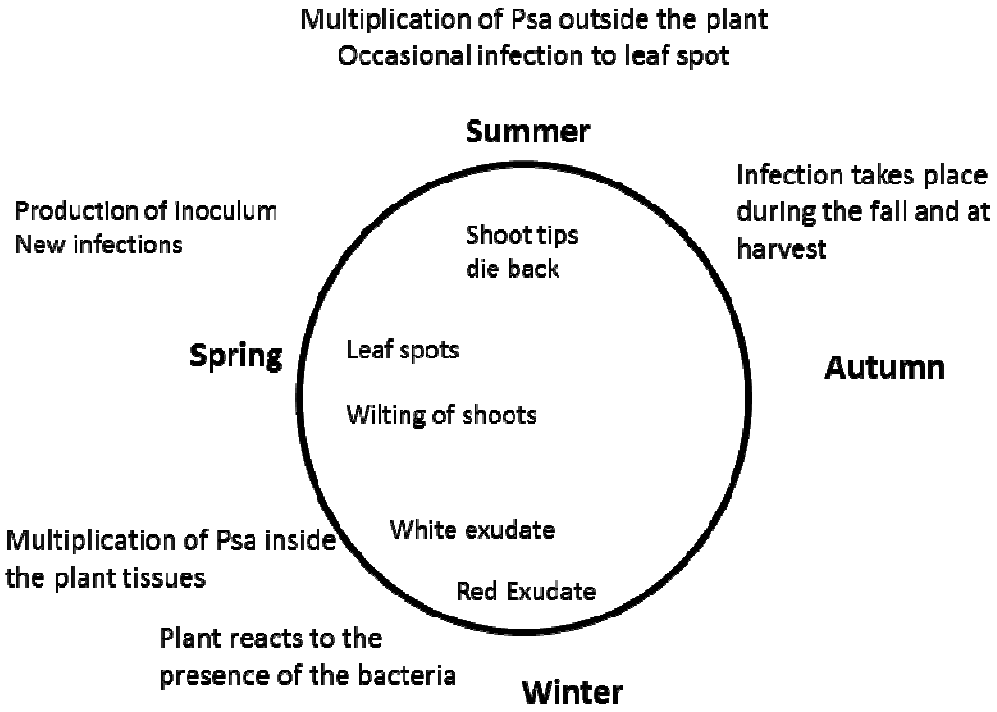

Figure 1 Hypothesized life cycle of *Pseudomonas syringae* pv. *actinidiae*, the causal agent of bacterial canker of kiwifruit (Vanneste et al, 2011).

4. Control measures

The most fundamental approach to the management of an unwanted disease is to ensure that it does not enter the country in the first place, by ensuring highly effective quarantine methods. This is obviously not applicable to New Zealand. Once established in a host population, control is difficult and options are limited for most bacterial diseases. There are currently no curative treatments for Psa, and all existing treatments are preventive measures. These include common bactericides like copper-based agrochemicals, disinfectants or sterilants, and antibiotics (Reglinski et al., 2013).

Serizawa et al., (1989) studied the spraying of streptomycin (200ppm) and kasugamycin (50ppm) antibiotics, and an inorganic copper (Cu) formulation [270ppm of $Cu(OH)_2$ with 95% $CaCO_3$] on 7-year-old kiwifruit vines in Japan, to test their efficacy in controlling Psa. After 3 applications at 7-day intervals, they found that the number of Psa affected leaves was reduced from 44.1% (Control) to 15% for the Cu treated vines and 4-7% for the antibiotic treated vines. The use of antibiotics for the control of plant pathogenic bacteria is legal in Asian Countries, but restricted or illegal in Europe and Australia. In New Zealand the use of antibiotics for control of plant pathogenic bacteria is restricted. Furthermore, there is a potential risk of the bacteria building resistance to the antibiotic as observed by Goto et al. (1994) and Han et al. (2003). Serizawa et al. (1989) also observed some phytotoxic symptoms of leaf cupping and marginal leaf chlorosis caused by streptomycin applications on kiwifruit vines.

Elicitors are products that induce the plant's defence mechanisms allowing it to fight infection. As the information available on the effectiveness of elicitors is limited, and the information projects benefits to be short term, these products have been recommended for use in addition to the copper treatments (Vanneste et al., 2011b).

The evidence for the effectiveness of biologicals for Psa control is poor (Balestra, 2007). Biological controls are outlined in a ZESPRI article (Brun & Max, 2012).

The spraying of copper-based agrochemicals is considered best practice in protecting against Psa (Vanneste et al., 2011b). Although their optimal use remains unknown, copper based bactericides play a major role in reducing the production of bacteria from cankers (Parker & Scarrow, 2011). Based on knowledge of the Psa outbreak in kiwifruit in New Zealand, Vanneste et al. (2011b) reported that it is highly recommended to spray copper compounds immediately after winter pruning, at bud break, and two and four weeks later (preferably before a major rain event). They also suggested that the post-flowering sprays should only be made in high risks situations, i.e., after

a major wind, rain or hail event. Later in the season, copper formulations are again recommended as a postharvest treatment and at leaf fall to prevent Psa from entering the vine through either picking wounds or leaf scars. Relatively high levels of copper sulphate are used by many growers to promote rapid and uniform leaf drop in autumn. Goodwin and McBrydie (2013) reported that the application of Cu-based agrochemicals during flower pollination significantly affected the kiwifruit yield.

A. Copper-based bactericides

The first commonly applied Cu solution, "Bordeaux mixture" ($CuSO_4.5H_2O$ and lime mixture), was first used in France in 1885, and has since been used extensively as a fungicide/bactericide (McBride et al., 1981). Copper forms complexes within pathogens, which destroy cell proteins and disrupts enzyme functions (Spencer-Phillips et al., 2002). In much of the world $CuSO_4$ is no longer recommended for use as it is highly soluble and toxic to the spray applicators and the environment (Mackie et al., 2012). Other less soluble copper formulations such as copper hydroxide ($Cu(OH)_2$) and copper oxychloride ($Cu_3Cl_2(OH)_4$), are now used preferentially. Most of the products used to date in New Zealand orchards are in the form of $Cu(OH)_2$ and CuO (Table 2). The use of Bordeaux mixture is restricted and is only to be used during the dormant seasons (Parker & Scarrow, 2011).

Table 2 Copper formulations on the ZESPRI Crop Protection Programme (Parker & Scarrow, 2011)

Timing	Product	Copper formulation
Pre-flowering	Copper sulphate	Copper sulphate
From harvest to flowering	Blue Shield DF	Copper hydroxide
	Cuprofix Disperss	Bordeaux mix
	Flo Bordo	Bordeaux mix
All season	AGPRO Cupric hydroxide	Copper hydroxide
	Champ DP	Copper hydroxide
	Champ Flo	Copper hydroxide
	Champ WG	Copper hydroxide
	Kocide Opti	Copper hydroxide
	Kocide 2000 DS	Copper hydroxide
	Mantissa Choice	Copper hydroxide
	Nordox 75 WD	Cuprous oxide
	Liquicop	Copper ammonium acetate

The effectiveness of commercial copper compounds depends on the formulation and concentration of copper salts used. For example, Balestra and Bovo (2003) demonstrated that $CuSO_4$ applied at a 10-fold lower concentration than a $Cu_3Cl_2(OH)_4$ formulation to kiwifruit vines effectively reduced the Psa bacteria count with 4 sprays, compared to 6 sprays of the latter.

Copper bactericides in the presence of lime would generally produce lower and more uniform concentrations of free copper, which in turn would be less likely to injure plant tissues (Alloway, 2008). For example with Bordeaux mix, which is the mixture of $CuSO_4$ and $Ca(OH)_2$ (lime), the hydroxide ions from the lime form a fixed Cu complex with the $CuSO_4$. However, there is no published evidence that adding lime to other Cu applications on fruit trees either reduces phytotoxicity, or extends the residual activity of the Cu. Brown et al. (1996) studied the effects of post-bloom applications of calcium hydroxide (3000 g /100 L) mixed with copper hydroxide (150 g Kocide@, 50% Cu /100 L) on the fruit quality of sweet cherry and apple. They reported that the application of a copper plus calcium mixture to cherries improved fruit resistance to cracking and firmness, and also improved the flesh firmness of apples. However, they did not study the pathogenic effect of these formulations. Ideally, Cu on the leaf surface should be at a high enough concentration to kill the bacteria but low enough not to cause injury to the plant. Possible plant injury may arise due to a lack of lime in a mixture; cold and wet weather conditions (time of application), and the application of excessive rates of Cu (Vanneste et al., 2011b).

B. Time of application

While it is necessary to ensure that efficient spray coverage is achieved when the vines are at risk, this has to be applied prior to key weather risk periods (Parker & Scarrow, 2011). The aim is to keep the number of sprays to a minimum while giving the best cover possible at times of high risk for infection. One of the main problems is related to the timing of sprays as the bactericide applications are not always applied at the appropriate time, for example due to unfavourable environmental conditions. Max et al. (2011a) stated that during summer, it is more important to apply copper after harvest to protect the stem wounds, as well as after the creation of any natural or man-made wounds. Growers can utilize the winter period between harvest and flowering to ensure the reduction of bacterium thus helping to prevent vascular infection. Based on this information Max et al. (2011a) have proposed a protocol for the use of protectant sprays (Table 3):

Table 3 Time to use copper-based bactericides

Orchards with no Psa infection and no high risk of Psa infection	Protective sprays are not required before harvest but good orchard hygiene would suggest applying after leaf fall and an application after pruning
Orchards with high risk of Psa infection (in or near the Te Puke Psa Priority Zone and/or a neighbouring block which has Psa infection	Protective spray use recommended in summer when infection is likely to occur. This should be followed by a spray immediately after the harvest, two leaf fall sprays and a winter spray
Psa confirmed	As above if the block is not to be cut back to a stump. For cut blocks, a copper before and after cutting is required. This should be followed up with regular protectant sprays before major rainfall events and after any event that creates vine damage e.g. hail or windstorm

Past experiences with copper-based sprays applied to kiwifruit orchards have shown occasional phytotoxic effects (Parker & Scarrow, 2011), excessive Cu accumulation in the soil and the occurrence of copper-resistant bacterial strains (Koh et al., 2012; Masami et al., 2004; Nakajima et al., 2002).

C. Copper resistant Psa

The efficacy of copper has been significantly reduced where copper-resistant strains of Psa have developed (Masami et al., 2004). To date Cu resistant strains of Psa have not been detected in New Zealand. Like other pathovars, the *Pseudomonas syringae* pv. *actinidiae* genome also includes sets of genes that are important for the survival of the bacterium or for competing with other micro-organisms (Scortichini et al., 2012). Indeed, the bacterium has enzymes involved in the inhibition of nitric oxide metabolism in the plants, namely nitric oxide dioxygenase and anaerobic nitric oxide reductase (Helmick et al., 2005). Nitric oxide plays a fundamental role in plant disease resistance by acting as a signal-inducing plant gene to synthesize defence-related compounds (Delledonne et al., 1998). The inhibition of nitric oxide synthesis consequently promotes the bacterial growth in plants. Further, copper ions are essential for bacterial species, but can induce toxic cellular effects if levels of free ions are not controlled (Cooksey, 1994).

Nakajima et al. (2002) found that the genetic and molecular basis of the copper resistance of *Pseudomonas syringae* pv. *tomato* in tomato was similar to copper resistance genes from *Pseudomonas syringae* pv. *actinidiae*. The copper resistant genes in tomato were identified as *cop operon* genes namely *Cop*A, *Cop*B, *Cop*C, and *Cop*D (Bender and Cooksey, 1986; Melano and Cooksey, 1988). The *Cop*A and *Cop*B are not responsible for copper resistance, but they are likely to be necessary for homeostasis and/or tolerance (Behlau et al., 2011), whereas *Cop*C and *Cop*D are responsible for increasing the strength of copper resistance of bacteria (Bondarczuk & Piotrowska-Seget, 2013). *Cop*A and *Cop*C are periplasmic proteins, and *Cop*B and *Cop*D are outer and inner membrane proteins (Cooksey, 1994). Nakajima et al. (2002) demonstrated that all strains isolated at the beginning of bacterial canker outbreaks in Japan in 1984 were copper sensitive with a minimum inhibitory concentration (MIC) of 0.75 mM $CuSO_4$. However, in 1987 and 1988 some strains isolated were copper resistant, with the MIC ranging from 2.25 to 3.0 mM. They also concluded that, with the repeated spraying of copper-based bactericides, the Psa showed the development of additional genes responsible for maximum resistance to copper, namely *Cop*R and *Cop*S, which were downstream from *Cop*D. Masami et al., (2004) identified that the mechanism of copper resistance in Psa consists of three different systems such as Cu-trapped by Cu-binding proteins, Cu-efflux mediated by a cation efflux protein, and Cu-transport mediated by a Cu-transporting ATPase. However, some studies observed that there was no development of Cu resistant Psa strains. For example, the Psa strain isolated from Italy during the 2008-09 outbreaks showed no resistance or tolerance to Cu (Ferrante & Scortichini, 2010). Vanneste and Voyle (2003) have made the initial steps towards identifying the presence of Cu resistant *Pseudomonas syringae* genes at a laboratory scale, and they have so far reported the possibility of future Cu resistant Psa strains in New Zealand. Prior

to this literature search, no studies had been published on the occurrence of Cu resistant Psa in New Zealand kiwifruit vines. KVH and Zespri are currently undertaking a monitoring programme to allow early detection of Cu-resistance in Psa strains in New Zealand.

D. Cu on plant surfaces

Menkissoglu and Lindow (1991a) showed that Cu^{2+} ions are the only form of copper that are toxic to copper-sensitive and copper-resistant strains of *P. syringae*. They found no evidence for the toxicity of copper when it forms complexes with glucose, fructose, sucrose, succinate, or citrate and organic compounds commonly found on leaf surfaces. In another experiment Menkissoglu & Lindow (1991b) conducted a field trial to determine the amount of total Cu and its fractions present on the surface of naval orange and beans leaves and their efficacy in controlling strains of *P. syringae* sprayed with various levels of Bordeaux mixture and $Ca(OH)_2$. They reported that up to 25% of the total copper applied via $Ca(OH)_2$ was deposited as dissolved copper on the leave surfaces of the naval orange. However, less than 0.1% of the dissolved copper on leaves was bio-available as Cu^{2+} ions, but it increased to a maximum concentration of approximately 100 µg Cu^{2+}/L about 10-20 days after the treatment. Interestingly, at 50 µg Cu^{2+}/L, no cells of the copper-sensitive *P. syringae* survived, but at least 10% of the initial copper-tolerant *P. syringae* strains survived on leaves containing 100 µg Cu/L as free Cu^{2+}. They also calculated the lethal concentration (LC_{50}) of Cu^{2+} to kill 50% of the *P. syringae* cell as an *in vitro* measurement. They reported that the maximum concentration of Cu^{2+} measured in citrus leaves were marginally less than the LC_{50} value for copper-tolerant *P. syringae* strains (100-300 µg Cu^{2+}/L); however, it was 30 times higher than the LC_{50} value for copper-sensitive *P. syringae* strains (10 µg Cu^{2+}/L). Furthermore, the concentrations of Cu^{2+} found on bean leaves treated with either high or low rates of $Cu(OH)_2$ were very similar. This demonstrates that the amount of Cu^{2+} will be largely determined by the equilibrium constants of the organic complexes and leaf surface chemistry, and not by the quantity of insoluble copper salts that are present (Adriano, 2001; Alloway, 1995; McBride et al., 1981).

Timmer and Zitko (1996) and Schwartz and McMillan (1989) found significant differences between copper hydroxide bactericides produced by different manufacturers, suggesting that inert materials could affect the availability of bio-available Cu^{2+}, even at equivalent rates of total Cu concentrations of the products. Scheck and Pscheidt (1998) demonstrated that the bio-available Cu^{2+} ions are the only predictors of formulation efficacy in reducing populations of copper-resistant and copper-sensitive strains of *Pseudomonas syringae* pv. *syringae* growing on tissue-cultured lilac and of copper-sensitive strains on field-grown lilac.

E. Cu Phytotoxicity

Excessive amounts of copper ions may cause damage to kiwifruit leaves and fruit. Copper caused phytotoxic symptoms such as the discolouration and cracking of stalks, silver-brown leaves, and the appearance of spots on the lower surfaces of the leaves in Japanese kiwifruit orchards (Serizawa et al., 1989). Most copper products are formulated to be almost insoluble in water at pH 7.0 (Menkissoglu & Lindow, 1991b). As the pH of the leaf surface or water decreases (below pH 6-7) the solubility of the copper bactericides increases and more copper ions are released. Further, both copper Psa control efficacy and Cu phytotoxicity are also dependent on the specific solubility of Cu formulations and on how finely the copper has been ground (Kiwifruit Vine Health, 2012, Table 4). Therefore, the concentration of Cu^{2+} on leaves depends on the equilibrium established with the complexed and soluble forms of copper (Fig. 2). It is essential to determine the appropriate combinations of copper products solubility, particle size and leaf pH to achieve better coverage and residual activity, and to avoid phytotoxicity.

Table 4 Solubility of copper formulations and their particle sizes

Cu formulations	Particle size (dia.) μ	Solubility
Copper oxide	1.0	Low ⇓ High
Copper oxychloride	1.8 - 3.1	
Copper ammonium complexes	0.3	
Copper hydroxide	2.5 - 3.1	
Copper sulphate	0.7 - 3.0	

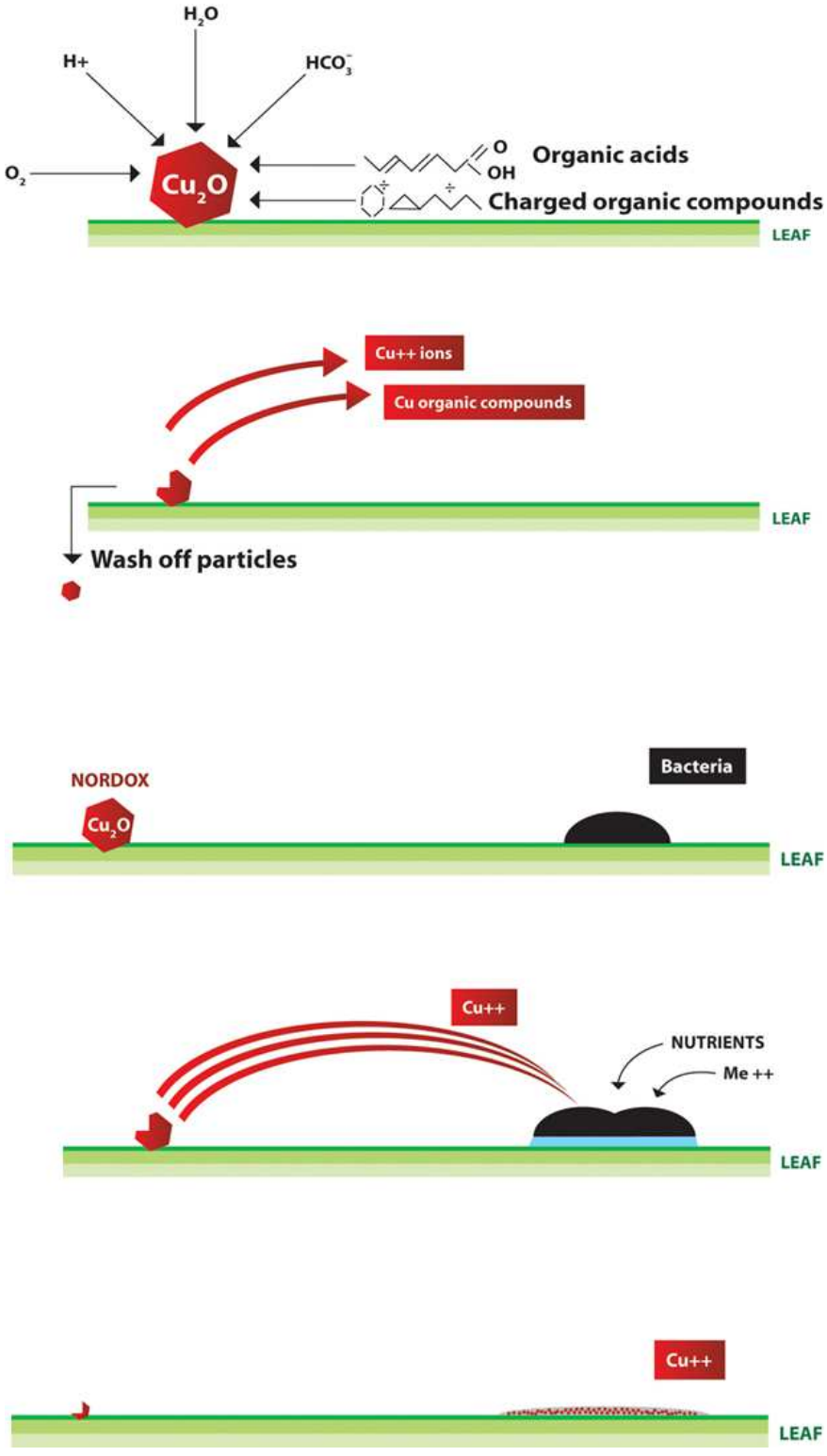

Figure 2 Schematic diagram for leaf chemistry (pH<6) and Cu based bactericidal effect (Torr, EastPack)

In New Zealand, studies conducted to identify phytotoxicity in kiwifruit orchards due to the usage of copper sprays are rare in the literature. However, there are some ongoing research projects which are reported by Kiwifruit Vine Health (KVH). Hawes (2012) commenced an experiment to study the phytotoxic effect of three commonly used Cu products namely Kocide™ (90 g/100L), Champ™ (75 g/100L), and Nordox™ (38 and 75 g/100L), sprayed 3-5 times during the post flowering period. The recent update of this trial showed that the application of these Cu products can cause light to moderate phytotoxicity (qualitative assessment only) in the leaves. They also showed that the frequency and concentration of the Cu sprays applied did not directly correlate with the level of phytotoxicity in the leaves, and also there was no phytotoxic effect observed on fruit. However, this research has failed to quantify the accumulation of Cu ions on the plant surface or within the plant cells, and any associated phytotoxic effects.

Max et al. (2011b) have commenced a study to determine the efficacy of injecting various copper formulations into Psa infected kiwifruit vines. In their recent update, they reported that the injection of Cu is not proving to be a success so far in the trial. The authors also did not observe any phytotoxic effects on the kiwifruit vines. Table 5 below explains some other ongoing trials investigating the phytotoxic effects of Cu based sprays on kiwifruit vines, in New Zealand.

There are no calculation tools available in literature for scheduling repeated kiwifruit orchard copper applications in relation to New Zealand climatic conditions. Beresford & Manktelow (1994) analysed the economically efficient Cu spray on apple and gained the results of seven field trials conducted over four seasons for black spot incidence. They described a regression equation model of better Cu spray management practices, for the efficient disease control and increased apple production. Dewdney et al. (2012) developed a web based ‘Cu decay curve’ tool to help citrus growers for the accurate and timely decision on copper applications in Florida.

Table 5 Ongoing trials investigating phytotoxic effects of Cu based sprays on kiwifruit vines in NZ

Location	Variety	Treatment	Time of Application	Results	Remarks	Reference
Gisborne	Gold 3	Kocide, Champ, Nordox (various rates and applications)	14 and 28 days after full bloom	No evidence of fruit damage associated with any treatment	None of the copper treatments caused fruit damage or yield	Lupton and Owen (2013)
Waikato	Hort16A Hayward	Kocide Champ Nordox (various rates)	Monthly for 3, 4 or 5 months	All copper treatments caused moderate leaf phytotoxicity No treatments caused fruit phytotoxicity effects.	None of the copper treatments caused severe leaf phytotoxicity	Hawes (2012)
French Orchards/NZ	Hort16A Hayward	Control Nordox 25g/100L (70% active Cu) Kocide 70g/100L (30% active Cu) Nordox 25g/100L + Agral 90 Kocide 70g/100L + Agral 90	Twice: Between 30 to 20 Days Before Flowering (DBF) Between 20 to 10 DBF	Leaf Cu content: 39-46 mg/kg in Hort16A, and 21-24 mg/kg in Hayward after 14 and 17 days after 2nd application, respectively	There were no significant phytotoxic effects on leaf or fruit development. Nordox and Kocide can be recommended at the rate ranging from 100 to 300g/ha Heavy rain-fall did not affect the adhesive ability of these chemicals	Brun and Max (2012)

Bay of Plenty	Hort16A	Dense Canopy – 2000L/ha Medium Canopy – 1500L/ha Light Canopy – 1000L/ha		For better spray coverage, the minimum required density is as follows: 25 Canes/bay, 4.1 mean leaf layer, 15% mean gap, 300 mm canopy depth	To effectively use Psa protectant chemicals, improved management to reduce canopy density is needed	Gaskin et al. (2012)
Plant Food Research (PFR)	Hayward	Nordox 75GW – 0.37g/L	1-5 days old flowers	The Cu spray significantly reduced fruit weights and seed number	Cu sprays may affect pollination of open flowers, especially of younger flowers. Cu sprays should not be applied immediately before carrying out artificial pollination	Goodwin and McBrydie (2013)
Hamilton	Hort16A	Various Cu products as recommended by KVH at the proposed rates Overhead irrigation was applied to ensure an infection period occurs	Start of the experiment	Minor leaf marking observed across Cu treatments. Lower rates of Nordox WG 75 effectively controlled Psa in Hort16A. Nordox at 25 and 37.5 g/100L reduced leaf spotting equally	The phytotoxicity differences between different Cu products and formulations are being investigated in a greenhouse study	Benge (2012)

Plant Protection Chemistry	Hort16A & Hayward Fruits	4x the recommended rates for each chemical: Nordox 75WG, Kocide Opti, Champ DP	Start of the experiment	Pre-rain residues ranged from 5-10 mg Cu/kg	These residues are well below the European Union toxic residue level for fruits of 20 mg Cu/kg	Jones (2011)
Plant Protection Chemistry	Hort16A & Hayward Leaves	Nordox 75WG, Kocide Opti , Champ DP	Start of the experiment	25 mm rainfall removed 50% of the initial Cu residues present on leaves Beyond 50 mm of rainfall, this dropped to 30%	Work is presently underway in France to determine the level of re-distribution of sprays on leaves	Jones (2011)
Plant Protection Chemistry	Hort16A & Hayward canes	Nordox 75WG, Kocide Opti , Liquicop , Cuprofix Disperss and Bordeaux (Various rates)	Start of the experiment	The Cu residues of the two varieties did not show any differences 100 mm of rainfall on the canes resulted in losses of 7 to 25% of initial Cu deposits	Bordeaux mix appeared to be the least affected by the rainfall and the Kocide Opti was the most affected	Jones (2011)

F. Cumulative copper in soil and its bioavailability

The repeated use of copper-based bactericides and fungicides to control horticulture plant diseases has led to long-term accumulation of Cu in the surface of some agricultural soils throughout the world (Mackie et al., 2012). For example, the repeated spraying of Bordeaux mixture in France to control vine downy mildew has resulted in a considerable build-up of total Cu concentrations in the topsoil, reaching values commonly ranging from 100 up to 1500 mg/kg ((Brun et al., 2001; Brun et al., 1998; Flores-Vélez et al., 1996). In New Zealand, Morgan and Taylor (2004) reported that the long term use of copper sprays in grape vineyards has resulted in Cu accumulations of up to 304 mg/kg soil over a period of 40 years. However, grape vines have rarely been reported to suffer from Cu phytotoxicity (Chaignon et al., 2003). Interestingly, Brun et al. (2001) showed that the concentrations of Cu in maize roots were very high (between 90 and 600 mg kg) when they were grown in contaminated vineyard soils where total Cu ranged from 38 to 251 mg/kg. In contrast, the Cu concentrations in the aerial plant parts remained as low as 18 mg/kg soil. Current guidelines in the NZ kiwifruit are to apply no more than 8 kg / ha / year of copper.

Copper can be present in both the solid and liquid phase of soils; and the dynamics of Cu in soil reactions are explained in Figure 3.

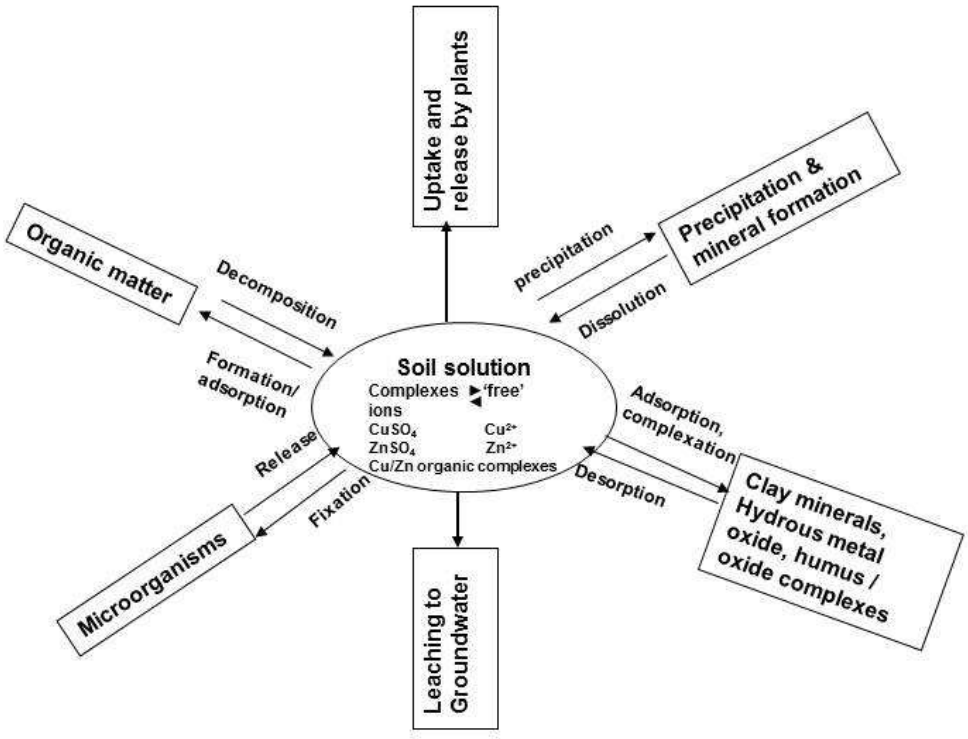

Figure 3 Dynamics of Cu reactions in soil (Adriano, 2001; Kabata-Pendias & Pendias, 2001; Loganathan et al., 2008)

The majority of Cu in soil solution phase forms complexes with dissolved organic carbon (DOC) and a small fraction is found as free copper ions (Jeyakumar et al., 2010a; McLaren & Clucas, 2001). This is because Cu forms very strong complexes with DOC through chelation with constituent functional groups such as carboxylic acids, amines and phenols (Altun & Köseoĝlu, 2005). Furthermore, the stability of these complexes is higher than those formed with other metals such as Zn (Pandey et al., 2000). Strobel et al. (2001) reported that Cu belongs to a group of elements that have strong interactions with DOC in the pH range 4 to 7.

Many studies have shown that the total metal content of a solid phase soil is usually not a good predictor of the metal concentrations in the plants (Jeyakumar et al., 2008; Jeyakumar et al., 2010b; McLaren & Clucas, 2001; McLaughlin et al., 2000). Copper can be associated with various soil components that differ in their ability to retain or release Cu. It forms complexes with organic matter; can be adsorbed onto the surfaces of clays, Fe and Mn oxides; is present in the lattice of primary silicate minerals or secondary minerals like carbonates, phosphates, sulphides; or occludes in amorphous materials (Jeyakumar et al., 2014; Alloway, 2005; Tessier et al., 1979). The bioavailability of copper in soils depends on the chemical properties of those soils that are likely to govern the fractionation of Cu, such as pH, redox potential, the content and nature of organic matter, clays and metal oxides and cation exchange capacity (McBride, 1981; Oliver et al., 2005; Ponizovsky et al., 2006). Solution phase Cu ions generally have a strong affinity with soil organic matter (SOM) (Stevenson, 1991). Therefore, the organic fraction in the soil can be the most important factor in determining Cu bioavailability (del Castilho et al., 1993). Pietrzak and McPhail (2004) mentioned that the conversion between copper fractions is slow, indicating that Cu can stay active in soils for many decades, and may result in leaching and transport to deeper soil layers. It has also been observed that as copper concentrations increase, the fraction bound to organic matter also increases (Fernández-Calviño et al., 2008). Morgan and Taylor (2004) identified the largest copper fraction in vineyards as copper residuals and organically bound copper closely followed by Fe bound copper. On the other hand, Pietrzak and McPhail (2004) reported that potentially available Cu (water soluble, sorbed and exchangeable fractions) in vineyard soils constitutes more than 60 % of total Cu in the upper part of soil profiles and the percentage decreased with increasing depth. Guinto et al. (2012) analysed the total Cu concentration of topsoils collected from 20 kiwifruit orchards in the Bay of Plenty and found that the mean Cu concentration did not exceed 35 mg/kg soil. However, the Cu concentration was significantly increased when they compared the Cu levels with 2009 samples collected from the same area. During this period the widespread use of copper-based sprays occurred due to the discovery of Psa. This literature search found that there hasn't

been any detailed research conducted to explain the Cu dynamics in soils in kiwifruit vineyards, especially in New Zealand.

5. Key points gathered from this literature review

A. High level research activities have been conducted on Psa strains and their resistant mechanisms in New Zealand and internationally, covering all major kiwifruit growing countries;
B. Cu resistant Psa studies are mainly focused on the micro or molecular biological aspects of the pathogen. The link between the resistant gene development mechanism and the role of bio-available Cu present on the plant surfaces is currently a major research gap that needs to be focused on;
C. There is a lack of research findings in the literature associated with the bioavailability of Cu^{2+} ions on the surface of kiwifruit vines, their efficacy in controlling Psa and their phytotoxic effects on kiwifruit vines;
D. Climatic factors influencing the effective control of Psa by Cu based bactericides and addition of buffering for slow release copper ions should be included as a part of the above studies mentioned;
E. Cu dynamics and the long-term effect of Cu residues and its accumulation in kiwifruit orchard soil have yet not been studied in detail.

6. References

Abelleira, A., Lopez, M. M., Penalver, J., Aguin, O., Mansilla, J. P., Picoaga, A., & Garcia, M. J. (2011). First Report of Bacterial Canker of Kiwifruit Caused by *Pseudomonas syringae* pv. *actinidiae* in Spain. *Plant Disease, 95*(12), 1583-1583. doi: 10.1094/pdis-06-11-0537.

Adriano, D. C. (2001). *Trace elements in terrestrial Environments: Biogeochemistry, bioavailability and risks of metals* (2nd ed.). New York: Springer.

Alloway, B. J. (1995). *Heavy metals in soils* (2nd ed.). Glasgow: Chapman and Hall.

Alloway, B. J. (2005). *Copper-deficient soils in Europe*. New York: International Copper Association.

Alloway, B. J. (2008). *Copper and zinc in soils: Too little or too much ?* Paper presented at the NZ Trace Elements Group Conference, Waikato University, Hamilton.

Altun, Y., & Köseoĝlu, F. (2005). Stability of copper(II), nickel(II) and zinc(II) binary and ternary complexes of histidine, histamine and glycine in aqueous solution. *Journal of Solution Chemistry, 34*, 213-231.

Balestra, G. M. (2007). Biocontrol of bacterial pathogens of kiwifruit plants. In A. R. Ferguson, E. W. Hewett, F. A. Gunson & C. N. Hale (Eds.), *Proceedings of the 6th International Symposium on Kiwifruit, Vols 1 and 2* (pp. 635-638).

Balestra, G. M., & Bovo, M. (2003). Effectiveness of copper compounds in the control of bacterial diseases of kiwifruit plants. *Acta Hortic., 610*, 399-402.

Balestra, G. M., Renzi, M., Ricci, L., Taratufolo, M. C., Quattrucci, A., Rossetti, A., & Mazzaglia, A. (2011). History of Kiwifruit Bacterial Diseases in Italy. In G. Costa & A. R. Ferguson (Eds.), *Vii International Symposium on Kiwifruit* (pp. 457-460).

Bastas, K. K., & Karakaya, A. (2011). First Report of Bacterial Canker of Kiwifruit Caused by *Pseudomonas syringae* pv. *actinidiae* in Turkey. *Plant Disease, 96*(3), 452-452. doi: 10.1094/PDIS-08-11-0675

Behlau, F., Canteros, B. I., Minsavage, G. V., Jones, J. B., & Graham, J. H. (2011). Molecular Characterization of Copper Resistance Genes from Xanthomonas citri subsp. citri and Xanthomonas alfalfae subsp. citrumelonis. *Applied and Environmental Microbiology, 77*(12), 4089-4096. doi: 10.1128/aem.03043-10

Benge, J. (2012). Testing of products for efficacy against Psa-V. *New Zealand Kiwifruit Journal-PSA Scientific Edition, March-April*, 28-29.

Beresford, R. M., & Manktelow, D. W. L. (1994). Economics of reducing fungicide use by weather-based disease forecasts for control of Venturia inaequalis in apples. *New Zealand Journal of Crop and Horticultural Science, 22*(2), 113-120. doi: 10.1080/01140671.1994.9513814

Bondarczuk, K., & Piotrowska-Seget, Z. (2013). Molecular basis of active copper resistance mechanisms in Gram-negative bacteria. *Cell Biology and Toxicology, 29*(6), 397-405. doi: 10.1007/s10565-013-9262-1

Brown, G. S., Kitchener, A. E., McGlasson, W. B., & Barnes, S. (1996). The effects of copper and calcium foliar sprays on cherry and apple fruit quality. *Scientia Horticulturae, 67(*3–4), 219-227. doi: http://dx.doi.org/10.1016/S0304-4238(96)00937-5

Brun, L. A., Maillet, J., Hinsinger, P., & Pépin, M. (2001). Evaluation of copper availability to plants in copper-contaminated vineyard soils. *Environmental Pollution, 111*(2), 293-302. doi: http://dx.doi.org/10.1016/S0269-7491(00)00067-1

Brun, L. A., Maillet, J., Richarte, J., Herrmann, P., & Remy, J. C. (1998). Relationships between extractable copper, soil properties and copper uptake by wild plants in vineyard soils. *Environmental Pollution, 102*(2-3), 151-161.

Brun, S., & Max, S. (2012). Do pre-flowering copper and elicitor applications affect vine performance? *Psa Scientific Edition, September-October*, 15-17.

Chaignon, V., Sanchez-Neira, I., Herrmann, P., Jaillard, B., & Hinsinger, P. (2003). Copper bioavailability and extractability as related to chemical properties of contaminated soils from a vine-growing area. *Environmental Pollution, 123*(2), 229-238. doi: http://dx.doi.org/10.1016/S0269-7491(02)00374-3

Chapman, J. R., Taylor, R. K., Weir, B. S., Romberg, M. K., Vanneste, J. L., Luck, J., & Alexander, B. J. R. (2012). Phylogenetic Relationships Among Global Populations of Pseudomonas syringae pv. actinidiae. *Phytopathology, 102*(11), 1034-1044. doi: 10.1094/PHYTO-03-12-0064-R

Chapman., J., Taylor, R., & Alexander, B. (2011). Second Report on Characterisation of *Pseudomonas syringae* pv. *actinidiae* (Psa) isolates in New Zealand. *Ministry of Agriculture and Forestry*.

Cooksey, D. A. (1994). Molecular mechanisms of copper resistance and accumulation in bacteria. *FEMS Microbiology Reviews, 14*(4), 381-386. doi: 10.1111/j.1574-6976.1994.tb00112.x

del Castilho, P., Chardon, W. J., & Salomons. W. (1993). Influence of cattle-manure slurry application on the solubility of cadmium, copper, and zinc in a manured acidic, loamy-sand soil. *Journal of Environmental Quality, 22*, 689-697.

Delledonne, M., Xia, Y., Dixon, R. A., & Lamb, C. (1998). Nitric oxide functions as a signal in plant disease resistance. *Nature, 394*, 585-588.Dewdney, M. M., Fraisse, C. W., Zortea, T., & Burrow, J. (2012). A Web-Based Tool for Timing Copper Applications in Florida Citrus. Retrieved 14 November, 2013, from http://edis.ifas.ufl.edu

Dye, D. W., Bradbury, J. F., Goto, M., Hayward, A. C., Lelliott, R. A., & Schroth, M. N. (1980). International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathogenic strains. *Review of Plant Pathology, 59*, 153-168.

EPPO. (2011a). First report of *Pseudomonas syringae* pv. *actinidiae* in Chile. *EPPO Rep. Serv. 3:2011/2055*.

EPPO. (2011b). First report of *Pseudomonas syringae* pv. *actinidiae* in Switzerland. *EPPO Rep. Serv. 8:2011/2168*.

Everett, K. R., Taylor, R. K., Romberg, M. K., Rees-George, J., Fullerton, R. A., Vanneste, J. L., & Manning, M. A. (2011). First report of *Pseudomonas syringae* pv. *actinidiae* causing kiwifruit bacterial canker in New Zealand. *Australian Plant Disease Notes, 6*(67-71).

Fernández-Calviño, D., Nóvoa-Muñoz, J. C., López-Periago, E., & Arias-Estévez, M. (2008). Changes in copper content and distribution in young, old and abandoned vineyard acid soils due to land use changes. *Land Degradation & Development, 19*(2), 165-177. doi: 10.1002/ldr.831

Ferrante, P., Fiorillo, E., Marcelletti, S., Marocchi, F., Mastroleo, M., Simeoni, S., & Scortichini, M. (2012). The importance of the main colonization and penetration sites of *Pseudomonas syringae* pv. *actinidiae* and prevailing weather conditions in the development of epidemics in yellow kiwifruit, recently observed in central italy. *Journal of Plant Path., 94*(2), 455-461.

Ferrante, P., & Scortichini, M. (2010). Molecular and phenotypic features of *Pseudomonas syringae* pv. *actinidiae* isolated during recent epidemics of bacterial canker on yellow kiwifruit (Actinidia chinensis) in central Italy. *Plant Pathology, 59*(5), 954-962. doi: 10.1111/j.1365-3059.2010.02304.x

Flores-Vélez, L. M., Ducaroir, J., Jaunet, A. M., & Robert, M. (1996). Study of the distribution of copper in an acid sandy vineyard soil by three different methods. *Etude de la distribution du cuivre dans un sol sableux acide de vignoble par trois methodes differentes, 47*(4), 523-532.

Gaskin, R., Manktelow, D., May, P., & Cook, S. (2012). How dense is too dense? The impact of canopy density on spray protection of vines. *NZ Kiwifruit Journal, September-October*, 57-60.

Goodwin, M., & McBrydie, H. (2013). The effect of copper on pollination. *New Zealand Kiwifruit Journal, January-February*, 52.

Goto, M., Hikota, T., Nakajima, M., Takikawa, Y., & Tsuyumu, S. (1994). Occurrence and properties of copper-resistance in plant pathogenic bacteria. *Annals of the Phytopathological Society of Japan, 60*, 147-153.

Guinto, D. F., Holmes, A., Rahman, H., & Rijkse, W. (2012). Trace element status of selected kiwifruit orchard topsoils in the Bay of Plenty. Paper presented at the Fertilizer and Lime Research Centre Workshop 2012, Palmerston North.

Han, H. S., Nam, H. Y., Koh, Y. A., Hur, J. S., & Jung, J. S. (2003). Molecular bases of high-level streptomycin resistance in Pseudomonas marginalis and *Pseudomonas syringae* pv. *actinidiae*. *Journal of Microbiology, 41*(1), 16-21.

Hawes, L. (2012). ZESPRI Hayward phytotoxicity trial update. *Kiwifruit Journal,* September-October, 52-55.

Helmick, R. A., Fletcher, A. E., Gardner, A. M., Gessner, C. R., Hvitved, A. N., Gustin, M. C., & Gardner, P. R. (2005). Imidazole Antibiotics Inhibit the Nitric oxide Dioxygenase Function of Microbial Flavohemoglobin. *Antimicrobial Agents and Chemotherapy, 49*(5), 1837-1843.

Holmes, A.W.; Taylor, R.; Miller, S.A.; Dean, F.; FitzGerald S.; and Surrey, M. (2013). Isolation and detection of Psa-V in kiwifruit green pollen and an evaluation of the ability of Psa-V to survive commercial pollen milling and extractions. Report to Zespri Innovation.

Jeyakumar, P., Loganathan, P., Anderson, C. W. N., Sivakumaran, S., & McLaren, R. G. (2014). Comparative tolerance of Pinus radiata and microbial activity to copper and zinc in a soil treated with metal-amended biosolids. Environmental Science and Pollution Research, 21(5), 3254-3263. doi: 10.1007/s11356-013-2271-z

Jeyakumar, P., Loganathan, P., Sivakumaran, S., Anderson, C. W. N., & McLaren, R. G. (2010b). Bioavailability of copper and zinc to poplar and microorganisms in a biosolids–amended soil. *Australian Journal of Soil Research, 48*(5), 1-11.

Jeyakumar, P., Loganathan, P., Anderson, C. W. N., Sivakumaran, S., & McLaren, R. G. (2010a, 1 – 6 August 2010). Response of Pinus radiata and soil microbial activity to increasing copper and zinc contamination in a soil treated with metal-amended biosolids. Paper presented at the 19th World Congress of Soil Science, Brisbane, Australia.

Jeyakumar, P., Loganathan, P., Sivakumaran, S., Anderson, C. W. N., & McLaren, R. G. (2008). Copper and zinc spiking of biosolids: effect of incubation period on metal fractionation and speciation and microbial activity. *Environmental Chemistry, 5*, 347–354. doi: 10.1071/EN08031.

Jones, V. (2011). Copper residues – are we under our fruit maximum residue limits (mrls) and what persistence are we getting on leaves? New Zealand Kiwifruit Journal, July-August, 1.6.

Kabata-Pendias, A., & Pendias, H. (2001). Trace elements in soils and plants. Boca Raton, Florida: CRC Press.

Kiwifruit Vine Health. (2012). KVH Information Sheet: Copper Spray Information. Retrieved 09 September, 2013, from http://www.kvh.org.nz/vdb/document/91314

Koh, J. K., Cha, B. J., Chung, H. J., & Lee, D. H. (1994). Outbreak and spread of bacterial canker in kiwifruit. *Korean Journal of Plant Pathology, 10*, 68-72.

Koh, Y. J., Kim, G. H., Jung, J. S., Lee, Y. S., & Hur, J. S. (2010). Outbreak of bacterial canker on Hort16A (Actinidia chinensis Planchon) caused by *Pseudomonas syringae* pv. *actinidiae* in Korea. *New Zealand Journal of Crop and Horticultural Science, 38*(4), 275-282. doi: 10.1080/01140671.2010.512624

Koh, Y. J., Kim, G. H., Koh, H. S., Lee, Y. S., Kim, S., & Jung, J. S. (2012). Occurrence of a New Type of *Pseudomonas syringae* pv. *actinidiae* Strain of Bacterial Canker on Kiwifruit in Korea. *The Plant Pathology Journal, 28*, 423-427.

Loganathan, P., Hedley, M. J., & Grace, N. D. (2008). Pasture soils contaminated with fertilizer-derived cadmium and fluorine: livestock effects. *Reviews of Environmental Contamination and Toxicology, 192*, 29-66.

Lupton, T., & Owen, M. (2013). Evaluating the fruit sensitive period to copper in Gold 3 kiwifruit. Report to ZESPRI International Ltd. pp 18.

Mackie, K. A., Müller, T., & Kandeler, E. (2012). Remediation of copper in vineyards – A mini review. *Environmental Pollution, 167*(0), 16-26. doi: http://dx.doi.org/10.1016/j.envpol.2012.03.023

Marcelletti, S., Ferrante, P., Petriccione, M., Firrao, G., & Scortichini, M. (2011). *Pseudomonas syringae* pv. *actinidiae* draft genomes comparison reveal strain-specific features involved in adaptation and virulence to actinidia species. *PLoS ONE, 6*(11), 1-17. doi: 10.1371/journal.pone.0027297

Marcelletti, S., & Scortichini, M. (2011). Clonal outbreaks of bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae* on A*ctinidia chinensis* and A*. deliciosa* in Italy. *Journal of Plant Pathology, 93*(2), 479-483.

Masami, N., Masao, G., Katsumi, A., & Tadaaki, H. (2004). Nucleotide Sequence and Organization of Copper Resistance Genes from *Pseudomonas syringae* pv. *actinidiae*. *European Journal of Plant Pathology, 110*, 223-226.

Max, S., ., Kay, C., Roger, G., Dallison, J., & Vanneste, J. L. (2011a). Getting one up on Psa. *Kiwifruit Journal,* March-April, 1-4.

Max, S., Kay, C., Benge, J., Stokes, C., Gentill, A., & Vanneste, J. L. (2011b). New Zealand injection trials update. *ZESPRI International*.

McBride, M., Tiller, K., & Merry, R. (1981). Copper in soils and plants. Sydney: Academic Press.

McBride, M. B. (1981). Forms and distribution of copper in solid and solution phases of soil. New York: Academic Press.

McLaren, R. G., & Clucas, L. M. (2001). Fractionation of copper, nickel, and zinc in metal-spiked sewage sludge. *Journal of Environmental Quality, 30*(6), 1968-1975.

McLaughlin, M. J., Hamon, R. E., McLaren, R. G., Speir, T. W., & Rogers, S. L. (2000). Review: A bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand. *Australian Journal of Soil Research, 38*(6), 1037-1086. doi: doi:10.1071/SR99128

Mellano, M. A., & Cooksey, D. A. (1988). Nucleotide sequence and organization of copper resistance genes from *Pseudomonas syringae* pv. *tomato*. *Journal of Bacteriology, 170*, 2879-2883.

Menkissoglu, O., & Lindow, S. E. (1991a). Relationship of free ionic copper and toxicity to bacteria in solutions of organic compounds. *Phytopathology, 81*, 1258-1263.

Menkissoglu, O., & Lindow, S. E. (1991b). Chemical forms of copper on leaves in relation to the bactericidal activity of cupric hydroxide deposits on plants. *Phytopathology, 81*, 1263-1270.

Morgan, R. K., & Taylor, E. (2004). Copper Accumulation in Vineyard Soils in New Zealand. *Environmental Sciences, 1*(2), 139-167. doi: 10.1080/15693430512331342602

Nakajima, M., Goto, M., & Hibi, T. (2002). Similarity between Copper Resistance Genes from *Pseudomonas syringae* pv. *actinidiae* and *P. syringae* pv. *tomato*. *Journal of General Plant Pathology : JGPP, 68*(1), 68-74.

Oliver, I. W., McLaughlin, M. J., & Merrington, G. (2005). Temporal trends of total and potentially available element concentrations in sewage biosolids: a comparison of biosolid surveys conducted 18 years apart. *Science of The Total Environment, 337*(1-3), 139-145.

Pandey, A. K., Pandey, S. D., & Misra, V. (2000). Stability constants of metal-humic acid complexes and its role in environmental detoxification. *Ecotox Environ Safe, 47*, 195-200.

Parker, B., & Scarrow, S. (2011). Copper use on kiwifruit vines. *Kiwifruit Journal,* May June, 1-3.

Pietrzak, U., & McPhail, D. C. (2004). Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia. *Geoderma, 122*(2–4), 151-166. doi: http://dx.doi.org/10.1016/j.geoderma.2004.01.005

Ponizovsky, A. A., Thakali, S., Allen, H. E., Di Toro, D. M., & Ackerman, A. J. (2006). Effect of soil properties on copper release in soil solutions at low moisture content: Wiley Periodicals, Inc.

Reglinski, T., Vanneste, J., Wurms, K., Gould, E., Spinelli, F., & Rikkerink, E. (2013). Using fundamental knowledge of induced resistance to develop control strategies for bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae*. *Frontiers in Plant Science, 4*. doi: 10.3389/fpls.2013.00024

Renzi, M., Copini, P., Taddei, A. R., Rossetti, A., Gallipoli, L., Mazzaglia, A., & Balestra, G. M. (2012a). Bacterial Canker on Kiwifruit in Italy: Anatomical Changes in the Wood and in the Primary Infection Sites. *Phytopathology, 102*(9), 827-840. doi: 10.1094/PHYTO-02-12-0019-R

Renzi, M., Mazzaglia, A., & Balestra, G. M. (2012b). Widespread distribution of kiwifruit bacterial canker caused by the European *Pseudomonas syringae* pv. *actinidiae* genotype in the main production areas of Portugal. *Phytopathologia Mediterranea, 51*(2), 402-409.

Rossetti, A., & Balestra, G. M. (2008). Pseudomonas syringae pv. syringae on Kiwifruit Plants: Epidemiological Traits and Its Control. In M. B. Fatmi, A. Collmer, N. Iacobellis, J. Mansfield, J. Murillo, N. Schaad & M. Ullrich (Eds.), *Pseudomonas syringae Pathovars and Related Pathogens – Identification, Epidemiology and Genomics* (pp. 65-68): Springer Netherlands.

Scheck, H. J., & Pscheidt, J. W. (1998). Effect of Copper Bactericides on Copper-Resistant and -Sensitive Strains of Pseudomonas syringae pv. syringae. *Plant Disease, 82*(4), 397-406. doi: 10.1094/PDIS.1998.82.4.397

Schwartz, H. F., & McMillan, M. S. (1989). Assaying bactericide efficacy against pseudomonads on bean leaf surfaces. *Plant Disease, 73*, 822-824.

Scortichini, M. (1994). Occurrence of *Pseudomonas syringae* pv. *actinidiae* on kiwifruit in Italy. *Plant Pathology, 43*, 1035-1038.

Scortichini, M., Marcelletti, S., Ferrante, P., Petriccione, M., & Firrao, G. (2012). *Pseudomonas syringae* pv. *actinidiae*: a re-emerging, multi-faceted, pandemic pathogen. *Molecular Plant Pathology, 13*(7), 631-640. doi: 10.1111/j.1364-3703.2012.00788.x

Serizawa, S., & Ichikawa, T. (1993a). Epidemiology of Bacterial Canker of Kiwifruit. 1. Infection and Bacterial Movement in Tissue of New Canes. *Annals of the Phytopathological Society of Japan, 59*(4), 452-459.

Serizawa, S., & Ichikawa, T. (1993b). Epidemiology of bacterial canker of kiwifruit. 2. The most suitable times and environments for infection on new canes. *Annals of the Phytopathological Society of Japan, 59*, 460-468.

Serizawa, S., & Ichikawa, T. (1993c). Epidemiology of bacterial canker of kiwifruit. 3. The seasonal changes of bacterial population in lesions and of its exudation from lesion. *Annals of the Phytopathological Society of Japan, 59*, 469-473.

Serizawa, S., & Ichikawa, T. (1993d). Epidemiology of bacterial canker of kiwifruit. 4. Optimum temperature for disease development on new canes. *Annals of the Phytopathological Society of Japan, 59*, 694-701.

Serizawa, S., Ichikawa, T., & Suzuki, H. (1994). Epidemiology of Bacterial Canker of Kiwifruit 5. Effect of Infection in Fall to Early Winter on the Disease Development in Branches and Trunk after Winter. *Annals of the Phytopathological Society of Japan, 60*(2), 237-244.

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. *Japanese Journal of Phytopathology, 55*(4), 427-436.

Spencer-Phillips, P. T. N., Gisi, U., & Lebeda, A. (2002). *Advances in downy mildew research* (Vol. 1). Dordrecht: Kluwer Academic Publishers.

Spiers, A. G. (2010). Management of *Pseudomonas syringae*. *Omnia Nutriology*, 1-6.

Stefani, E., & Giovanardi, D. (2011). Dissemination of *Pseudomonas syringae* pv. *actinidiae* through pollen and its epiphytic life on leaves and fruits. *Phytopathol. Mediter, 50*, 489-496.

Stevenson, F. J. (1991). Organic matter-micronutrient reactions in soil. In J. J. Mortvedt (Ed.), *Micronutrients in agriculture. SSSA Book Series number 4* (pp. 145-186). Madison, WI: Soil Science Society of America Inc.

Stokes, C. (2013). *Overcoming challenges in supporting remote and regional growers*. Paper presented at the APEN International Conference 2013-Transformative change: Chosen or unchosen, Christchurch.

Strobel, B. W., Hansen, H. C. B., Borggaard, O. K., Andersen, M. K., & Raulund-Rasmussen, K. (2001). Composition and reactivity of DOC in forest floor soil solutions in relation to tree species and soil type. *Biogeochemistry, 56*, 1-26.

Takikawa, Y., Serizawa, S., Ichikawa, T., Tsuyumu, S., & Goto, M. (1989). *Pseudomonas syringae* pv. *actinidiae* pv. nov.: the causal bacterium of canker of kiwifruit in Japan. *Annals of the Phytopathological Society of Japan, 55*, 437-444.

Tessier, A., Campbell, P. G. C., & Bisson, M. (1979). Sequential extraction procedure for the speciation of particulate trace metals. *Analytical Chemistry, 51*(7), 844-851. doi: 10.1021/ac50043a017

Timmer, L. W., & Zitko, S. E. (1996). Evaluation of copper fungicides and rates of metallic copper for control of melanose on grapefruit in Florida. *Plant Disease, 80*, 166-169.

Torr, T. (unknown). Psa: Understanding how coppers works. Retrieved 19 January, 2014, from http://www.eastpack.co.nz/vdb/document/13931

Vanneste, J. L. (2012). *Pseudomonas syringae* pv. *actinidiae* (Psa): a threat to the New Zealand and global kiwifruit industry. *New Zealand Journal of Crop and Horticultural Science, 40*(4), 265-267. doi: 10.1080/01140671.2012.736084

Vanneste, J. L., Giovanardi, D., Yu, J., Cornish, D. A., Kay, C., Spinelli, F., & Stefani, E. b. (2011a). Detection of *Pseudomonas syringae* pv. *actinidiae* in pollen samples. *NZ Journal of Plant Protection*(64), 246-251.

Vanneste, J. L., Kay, C., Onorato, R., Yu, J., Cornish, D. A., Spinelli, F., & Max, S. (2011b). Recent advances in the characterisation and control of *Pseudomonas syringae* pv. *actinidiae*, the causal agent of bacterial canker on kiwifruit. *Acta Hortic., 913*(Proceedings of the VIIth International Symposium on Kiwifruit, 2010), 443-455.

Vanneste, J. L., Poliakoff, F., Audusseau, C., Cornish, D. A., Paillard, S., Rivoal, C., & Yu, J. (2011c). First Report of *Pseudomonas syringae* pv. *actinidiae*, the Causal Agent of Bacterial Canker of Kiwifruit in France. *Plant Disease, 95*(10), 1311-1312. doi: 10.1094/pdis-03-11-0195

Vanneste, J. L., & Voyle, M. D. (2003). Genetic basis of copper resistance in NZ strains of *Pseudomonas syringae*. In S. M. Zydenbos (Ed.), *NZ Plant Protection,* Vol. 56, pp. 109-112.

Vanneste, J. L., Yu, J., Cornish, D. A., Tanner, D. J., Windner, R., Chapman, J. R., . . . Dowlut, S. (2013). Identification, Virulence, and Distribution of Two Biovars of *Pseudomonas syringae* pv. *actinidiae* in New Zealand. *Plant Disease, 97*(6), 708-719. doi: 10.1094/PDIS-07-12-0700-RE

Young, J. M., Dye, D. W., Bradbury, J. F., Panagopoulos, C. G., & Robbs, C. F. (1978). A proposed nomenclature and classification for plant pathogenic bacteria. *New Zealand Journal of Agricultural Research, 21*(1), 153-177. doi: 10.1080/00288233.1978.10427397.