OPTIMISING COPPER SPRAYS ON KIWIFRUIT: A REVIEW

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Abstract
The spraying of copper-based bactericides is one of the most effective practices in protecting against Psa. Although their use has yet to be optimised, copper-based bactericides play a major role in reducing the production of spores from the cankers. In New Zealand, copper compounds have been recommended for spraying immediately after winter pruning, at bud break, two and four weeks after bud break, and in high risk situations like after a major wind, rain or hail event. Some studies have reported that the application of Cu-based bactericides significantly affects the kiwifruit yield. However, studies on the most effective system for application of these Cu-based agrichemicals have been limited. In addition, there have been no studies published on the occurrence of Cu resistant Psa in Kiwifruit vines. This review of literature has identified the research gap that is needed to be addressed immediately for the effective control of Psa and the occurrence of Cu resistant Psa in Kiwifruit vines.

***Pseudomonas syringae* pv. *actinidiae* (Psa)**
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 official report 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 adapt an efficient kiwifruit management plan for a Psa affected environment. This includes effective control of Psa without compromising the quality of kiwifruit orchards, potentially growing Psa-resistant varieties and understanding how climate factors affect vine susceptibility (Vanneste, 2012).

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. However, 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 were 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. However, in Europe, Australia and New Zealand, the use of antibiotics for control of plant pathogenic bacteria is restricted or illegal. 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 the streptomycin spray 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., 2011).

Biological controls reference to ZESPRI (Brun and Max, 2012), the effectiveness of biologicals for Psa control is poor (Balestra, 2007).

The spraying of copper-based agrochemicals is considered best practice in protecting against Psa (Vanneste et al., 2011). 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. (2011) 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. However, Goodwin and McBrydie (2013) reported that the application of Cu-based agrochemicals during flower pollination significantly affected the kiwifruit yield.

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 disrupt 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 1). The use of Bordeaux mixture is restricted and is only to be used during the dormant seasons (Parker & Scarrow, 2011).

The effectiveness of the commercial copper compounds depends on the formulation and concentration of copper salts used (Balestra and Bovo 2003). 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). 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., 2011).

Table 1. 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

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 protect the leaves after harvest, any natural or man-made wounds. Growers can utilize the winter period between harvest and flowering to ensure the reduction of bacterium over winter and thus help to prevent vascular infection. Based on this information Max et al. (2011a) have proposed a protocol for the use of protectant sprays (Table 2):

Table 2: Time to use the Cu 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 a winter after pruning
Orchards with high risk of Psa infection (in or near the Psa Priority Zone)	Protective spray use recommended in summer. 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. For cut blocks, a spray before and after cutting is required. This should be followed up with regular protectant sprays before major rainfall events and after hail or windstorm

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

Cu-resistant Psa

The efficacy of copper has been significantly reduced by the occurrence of copper-resistant strains of Psa (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 pathogen can inhibit the nitric oxide metabolism of the plant. Nitric oxide plays a fundamental role in plant disease resistance by acting as a signal-inducing plant gene to synthesize defence-related compounds. The inhibition of nitric oxide synthesis by Psa consequently promotes the bacterial growth in vines (Delledonne et al., 1998). 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). 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 (Ferrante & Scortichini, 2010). Prior to this literature search, no studies had been published on the occurrence of Cu resistant Psa in New Zealand kiwifruit vines. 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. KVH and Zespri are currently undertaking a monitoring programme to allow early detection of Cu-resistance in Psa starins in New Zealand.

Cu on plant surfaces

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). Menkissoglu and Lindow (1991a) showed that Cu^{2+} ions are the only form of copper that is 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, only less than 0.1% of the dissolved copper on leaves was bioavailable as Cu^{2+} ions, but it increased to a maximum concentration of approximately 100 µg Cu^{2+}/L after about 10-20 days following 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 bioavailable Cu^{2+}, even at equivalent rates of total Cu concentrations of the products. Scheck and Pscheidt (1998) demonstrated that the bioavailable 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.

Cu phytotoxicity

In New Zealand, studies conducted to identify the phytotoxicity in kiwifruit orchards due to the usage of copper sprays, are rare in literature. However, there are some ongoing research projects which are reported by Kiwifruit Vine Health (KVH). Hawes (2012) has recently commenced an experiment to study the phytotoxic effect of 3 commonly used Cu products namely Kocide^{TM} (90 g/100L), Champ^{TM} (75 g/100L) and Nordox^{TM} (38 and 75 g/100L) sprayed at 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 the 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 fruits. 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 3 below explains some other ongoing trials investigating the phytotoxic effects of Cu based sprays on kiwifruit vines, in New Zealand.

Table 3: Selected on-going trials on investigating the phytotoxic effects of Cu based sprays on kiwifruit vines, in New Zealand

Location	Variety	Treatment	Time of Application	Results	Remarks	Reference
Gisborne	Gold 3	Kocide, Champ, Nordox (various levels 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 levels)	Monthly for 3, 4 or 5 months	All copper treatments caused leaf phytotoxicity to a moderate level. The treatments did not cause fruit phytotoxicity effects.	None of the copper treatments caused severe leaf phytotoxicity.	Hawes (2012)
French Orchards/NZ	Hort16A Hayward	Control Nordox 25g/100L (75% active Cu) Kocide 70g/100L (35% 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.	Copper sprays may affect the pollination of any open flowers especially on the 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 the Cu treatments. Lower rates of Nordox WG 75 effectively controlled Psa in Hort16A. Nordox at the rates of 25 and 37.5 g/100L reduced leave spotting equally.	The phytotoxicity differences between different Cu products and formulations are being investigated.	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 were 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)

Cumulative copper in soil and its bioavailability

The repeated use of copper-based bactericides/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 copper spray in vineyards has resulted in Cu accumulations of up to 304 mg/kg soil, over a period of 40 years. However, the 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 the 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 parts remained as low as 18 mg/kg soil.

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 1.

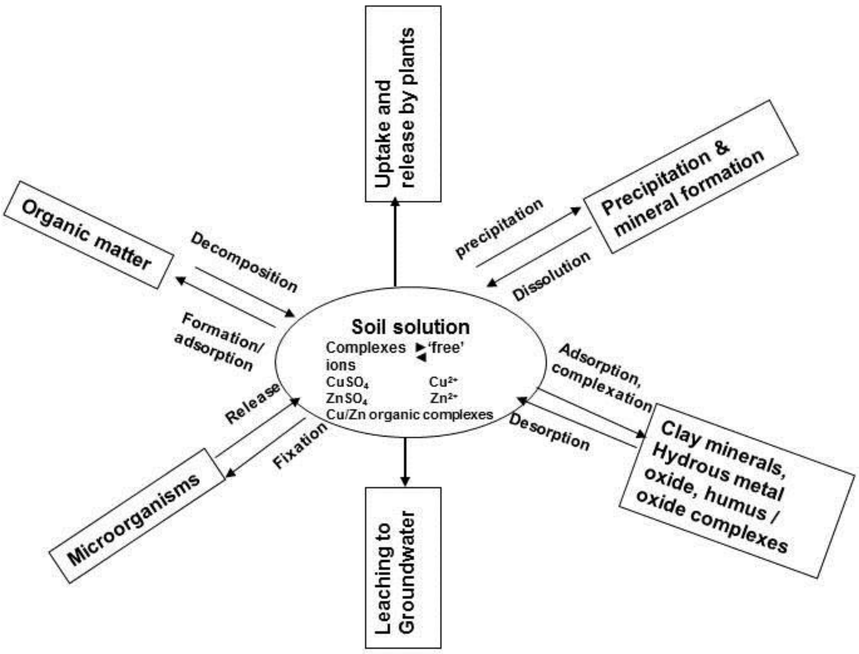

Figure 1: Dynamics of Cu reactions in soil (Adriano, 2001; Kabata-Pendias & Pendias, 2001; Loganathan et al., 2008)

The majority of Cu in soil solution phase is it forms complexes to dissolved organic carbon (DOC) and a small fraction is found as free copper ions (Jeyakumar et al., 2010a; Jeyakumar et al., 2014; 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 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, adsorbed onto the surfaces of clays, Fe and Mn oxides, present in the lattice of primary silicate minerals or secondary minerals like carbonates, phosphates, sulphides, or occluded in amorphous materials (Alloway, 2005; Tessier et al., 1979). The bioavailability of the 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 long periods of time, greater than tens of years, 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 Bay of Plenty, New Zealand 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. With this literature search I found that there hasn't been any detailed research conducted to explain the Cu dynamics in soils in kiwifruit vineyards, especially in New Zealand.

Key points gathered from this literature review

- High level research activities have been conducted on Psa strains and their resistant mechanisms at both New Zealand and international levels, covering all major kiwifruit growing countries.
- The 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 bioavailable Cu present on the plant surfaces is currently a major research gap that needs to be focused on.
- There is a lack 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.
- The climatic factors influencing the effective control of Psa by Cu based bactericides, should be included as a part of the above studies mentioned.
- The Cu dynamics and the long term effect of Cu residues and its accumulation in the kiwifruit orchard soil system have yet not been studied in detail.

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