PlusGroup
RESEARCH DIVISION

ZESPRI Innovation Project VI1557

SFF Copper – Effects of 'other' products on copper ion release: Final Report

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Executive summary

Various copper formulations have proven to be effective at controlling the bacterial vine disease Psa-V and bioefficacy has been shown to be related to the free cupric (Cu^{2+}) ion that is released from copper deposits into solution on leaf surfaces.

Other foliar sprays commonly used on kiwifruit orchards may influence the release of copper ions (Cu^{2+}) and alter the chemistry of the leaf surface in a way that either results in an increase or reduction in Cu^{2+} release. Reductions in the available pool of Cu^{2+} will result in reduced bio-efficacy and plant protection. Conversely, the release of too many copper ions may increase the potential for copper-induced phytotoxicity.

The objectives of this two-year study were to determine the extent to which other foliar spray products interact with copper and influence the release of Cu^{2+} on leaf surfaces.

Results showed that under the conditions used in these trials a number of products interacted with copper which resulted in an increase or decrease in available Cu^{2+} which sometimes varied according to copper formulation. The key findings were as follows:

- Citrox BioAlexin™ consistently increased the concentration of Cu^{2+} on plant leaf surfaces when applied after copper.
- Consistent reductions in Cu^{2+} release were observed when Acadian was applied prior to, as a tank mix, or post Nordox® and Kocide® applications.
- The effects of Kasumin™ on Cu^{2+} release were very inconsistent, especially with Kasumin-Kocide interactions. However, when Kasumin™ was applied as a tank mix with Kocide® onto an orchard canopy, an increase in Cu^{2+} release on leaves was observed over time.
- KeyStrepto™ had the effect of greatly reducing copper availability in solution with Nordox® and Coptyzin. The availability of Cu^{2+} on leaves was also significantly reduced when KeyStrepto™ was applied 24hrs prior to Nordox®.
- Although Actigard™ when mixed with three different copper formulations in solution resulted in a reduction in Cu^{2+}, the availability of Cu^{2+} significantly increased when applied with Nordox® on leaves. However, as this product was not tested on leaves in combination with Kocide® and Coptyzin, the disparate results with Nordox® are difficult to explain. Further trial work may be required in order to validate these results.

This study highlights the need for potential interactions with other products to be considered when planning an orchard spray operation so that product efficacy is not compromised.

1. Introduction

Copper spray formulations are routinely used on kiwifruit orchards as a protectant against the bacterial vine disease *Pseudomonas syringae pv. actinidiae* (Psa). The anti-microbial properties of copper have been known for centuries. As a result of numerous studies (e.g., Dumestre *et al.*, 1999; Luo *et al.*, 2006) it has now been determined that it is predominantly the free cupric (Cu^{2+}) ion rather than other forms of copper, such as soluble or complexed, that is toxic to microorganisms, including Psa.

After a copper compound has been applied to a canopy the bioavailable form of free ionic copper is steadily released into solution on leaf surfaces when there is moisture available. There are a range of different copper products on the market and the solubility and associated release of Cu^{2+} in solution and on plant surfaces depends on the chemical formulation of each. Commonly used copper products increase in solubility in the following order: chelated copper (e.g, Coptyzin) → Copper oxides (e.g., Nordox®) → Copper oxychlorides → copper ammonium complexes (e.g, Liquicop) → copper hydroxides (e.g., Kocide® Opti™, Champ®) → copper sulphate. The potential efficacy of these copper formulations are also related to leaf physiology and environmental factors. Organic compounds present on leaf surfaces react with copper deposits and copper ions may either be released or bound into non-available organic complexes. The availability of bio-effective copper ions is mostly determined by the equilibrium constants of the organic copper complexes and leaf surface chemistry, and not by the total concentration of copper present on the leaf surface (Adriano, 2001; Alloway, 1995; McBride et al., 1981). A study on the complexation of copper by organic solutes found that the toxicity of copper to strains of both copper-sensitive and copper-tolerant *Pseudomonas syringae* was reduced by up to 30 X when copper was in solution with organic compounds such as glucose, fructose and citrate (Menkissoglu & Lindow, 1991).

Other foliar sprays commonly used on kiwifruit orchards could influence the release of copper ions and alter the chemistry of the leaf surface in a way that either results in an increase or reduction in Cu^{2+} release. Reductions in the available pool of Cu^{2+} will result in reduced bio-efficacy and plant protection. Conversely, the release of too many copper ions may increase the potential for copper-induced phytotoxicity.

2. Objectives and Aims

The objectives of this two year study were to determine the effect of commonly used foliar agrichemical products on the release of copper ions in tank mix solutions and on kiwifruit leaf surfaces as follows:

1. Laboratory screening trial (Year 1) – To measure the concentration of Cu^{2+} as released in combinations of solutions comprising 3 different copper formulations and a range of commonly used foliar sprays, with differing chemical compositions.
2. Greenhouse trial (Year 1) – To measure Cu^{2+} released on leaves of potted G3 clonal plants when sprayed with each of three copper formulations or a combination of copper and 'other' spray formulations as selected based on the initial screening trial.
3. Greenhouse trial (Year 2) – To measure the release of Cu^{2+} on leaves of potted G3 clonal plants when sprayed with two commonly used copper formulations or combinations of copper and 'other' spray formulations. An extension of the Year 1 trial which included an assessment of how the timing of application of 'other' products in relation to copper applications may influence Cu^{2+} release.
4. Orchard Trial (Year 2) – To measure the release of Cu^{2+} on orchard vine leaves when commercially sprayed with one copper formulation and a combination of copper and one 'other' spray formulation. This would also enable the comparison of results from potted plants and orchard vines of the same variety.

During the course of the Year 2 trial, further investigations were carried out in order to provide information on three specific queries related to the trial methodology:

Q1. What effect does re-wetting of the leaf surface have on Cu^{2+} release?

The main greenhouse trials did not include water spray controls. Therefore, it was deemed necessary to determine if the measured increase in Cu^{2+} release when other products were applied after copper was solely due to the product itself or the effect of re-wetting.

Q2. Do interactive products need to be left for a longer period of time on the leaf surface to reach an equilibrium prior to sampling?

The sampling of leaves for all experiments in Year 2 was undertaken once leaves were fully dry. For previous experiments carried out in Year 1, leaves were sampled 24hrs or 48hrs after product application. It was hypothesized that a longer period of time prior to sampling may be required in order for products to reach an equilibrium on leaf surfaces.

Q3. What effect do environmental conditions have on product interactions and Cu^{2+} release (e.g., greenhouse vs. orchard)?

The majority of trials on product-copper interactions were undertaken in a greenhouse in order to maintain a controlled environment and prevent product wash-off and physical damage. This sub-trial was conducted in order to determine how copper persistence and Cu^{2+} release may be influenced by the environment in which the trial plants are located and the conditions to which they are subjected to (i.e. wind, dew, sunlight, rain).

3. Methods

Year 1

A range of foliar agrichemicals commonly used on kiwifruit orchards were identified. These were selected so as to incorporate a range of differing chemical formulations, such as adjuvants, foliar fertilisers, and elicitors. An initial laboratory-based rapid screening test was developed and carried out to determine the effect of these formulations on Cu^{2+} release when mixed in solution with three different copper formulations. Based on the results of the initial study, selected formulations were chosen to determine copper ion release from Nordox® on leaves of potted G3 kiwifruit plants in a greenhouse environment.

Rapid screening laboratory trial

A range of foliar sprays were prepared at label rates in solution with label rates of Nordox® Kocide® Opti™ or Coptyzin (Table 3.1).

Table 3.1: Foliar sprays used in the trial

Product	Product Description	Active compounds	Rate (g-mL/L)
Nordox®	copper bactericide	copper oxide	0.375
Kocide® Opti™	copper bactericide	copper hydroxide	0.7
Coptyzin	copper bactericide	copper sulphate, zinc sulphate, Tetraethylenepentamine, Triethylentetramine	0.15
Actigard™	elicitor	acibenzolar-s-methyl	0.2
Du-Wett®	adjuvant	trisiloxane ethoxylate surfactant	0.25
WETCIT®	adjuvant	boric acid/orange oil surfactant	3.0
Urea	foliar fertiliser	nitrogen compounds	10
KeyStrepto™	bactericide	Streptomycin sulphate and surfactant	0.6
Citrox BioAlexin™	elicitor	citrus extract, glycerine, palm oil, water	1.5
Hi-Cane®	bud break enhancer	Hydrogen cyanamide, phosphoric acid	60
Ambitious™	elicitor	Forchlorfenuron cytokinin	0.75
Acadian	elicitor	seaweed extract	1.0
Proclaim®	insecticide	Emamectin benzoate	0.02

A cupric ion selective electrode (ISE) (Orion *Sureplus ion flow* combination) coupled with a benchtop meter (Cyberscan 2100) was used to determine the relative millivolt potential (mV) of each solution (Figure 3.1). A lower millivolt reading corresponds to a lower Cu^{2+} concentration. For relatively high mV readings the ionic strength of each sample solution was adjusted with 5M KNO_3 prior to measurement to enable stable and accurate readings to be taken. Calibration curves produced from standard copper solutions in the range of 0.1 – 1000 ppm and 0.001 - 0.662 ppm were used to determine the concentration values of Cu^{2+} in each solution. For solutions with low levels of Cu^{2+}, measurements were made according to the methods of Sauvé (1995) and Adveef *et al.* (1983). The ionic strength of each sample solution was adjusted with 1M KNO_3 prior to measurement. A standard calibration curve was prepared from a range of buffered copper standards covering the range of sample concentrations where the pCu^{2+} of each standard was calculated from known stability constants and the pH of each standard. Copper standards were prepared using $Cu(NO_3)_2$ and ethylenediamine (EN) and the pH was adjusted with HNO_3 to achieve nominal pH values of 4, 5, 6, 7 and 9. The concentration of Cu^{2+} in solution was calculated based on pCu^{2+} values, where: $pCu^{2+} = -\log [Cu^{2+} \text{ activity}]$.

The temperature of wash solutions remained constant throughout each experiment (23 - 24°C) and the pH of each solution after the addition of ISA was measured at the time of ISE readings with a flat end glass electrode (Metrohm) calibrated with pH 4 and 7 standard buffer solutions.

Figure 3.1: Cupric ISE Cu^{2+} measurement setup

Potted plant greenhouse trial

Greenhouse setup and spray applications

One copper formulation (Nordox®) and one kiwifruit variety (G3) were used in this trial which was conducted in a plastic greenhouse. Plants were assigned to two groups, with one group receiving a fine misting in the early morning in order to simulate dew formation in an orchard situation and the other adjacent group receiving no misting (Figure 3.2).

Products for combination spray applications were selected based on the results of the laboratory trial. Leaves were sprayed to runoff on the upper and lower surfaces of leaves with a 1L plastic bottle fitted with a trigger sprayer. Products were applied at standard rates either 24hrs before, in a mixture with copper or 24hrs post copper application (Table 3.2). Each treatment was applied to three plants. Du-Wett® was applied with Nordox® for each combination treatment. Spray applications were carried out between 9 and 10am.

Figure 3.2: Plant misting setup

Table 3.2: Potted plant spray regime

Copper formulation	Additional Product(s)	Timing of application	'Other' product rate (g-mL/L)	Date applied
*Nordox®	-	-		18 March
Nordox®	Du-Wett®	tank mix	0.25	18 March
Nordox®	Du-Wett® + Actigard™	tank mix	0.2	18 March
Nordox®	Acadian	24hr prior, tank mix, and 24hr after copper	1.0	17, 18, 19 March
Nordox®	Du-Wett®	tank mix		31 March
Nordox®	KeyStrepto™	24hr prior and 24hr after copper	0.6	30 March, 1 April
Nordox®	Citrox BioAlexin™	24hr prior and 24hr after copper	1.5	30 March, 1 April

*Nordox® was used at summer rate, 37.5g/100L. Du-Wett® added to mix when Nordox® was applied with other products.

Sampling and analysis

Eight leaves from three plants in each treatment were sampled for copper analysis 48hrs after copper spray applications. Leaves (excluding the petiole) were directly transferred from the plant into plastic sealable bags and transported immediately to the laboratory for analysis. Leaf temperatures were measured with an infrared thermometer (Optics MS) at the time of sampling. Leaf temperatures were measured in the laboratory for those leaves sampled on 20 March.

In the laboratory the leaf surface pH of each leaf sampled on 20 March was measured with a flat end glass electrode (Metrohm). Two to three measurements were made per leaf and the results averaged. Leaves were then washed in the bags with 50 mL of deionised water. This was achieved by placing the bag and enclosed leaf flat on an orbital shaker set at 230 rpm for 2 mins each side so that the entire leaf surface was in contact with the water. The supernatant was then decanted into a plastic measuring cup and readings taken with the cupric ISE according to the method described above. Each leaf was photographed and the total leaf area for each leaf determined using the software programme Image J. The concentration of Cu^{2+} cm^{-2} of leaf surface was calculated based on pCu^{2+} and leaf area values.

Phytotoxicity monitoring

Three leaves per treatment were tagged with coloured wool and photographed periodically for 12-16 days in order to monitor visual symptoms of phytotoxicity.

Statistical analysis

Data were analysed by one way analysis of variance (ANOVA) using STATSTICA v12 (StatSoft) and Microsoft Excel t-tests assuming unequal variances. Comparison of means was undertaken using Tukey's honestly significant difference (HSD) post hoc test.

Year 2

Potted plant greenhouse trial

<u>Greenhouse setup and spray applications</u>

Potted clonal G3 plants grafted on Bruno rootstocks were used in the trial. Initially plants were positioned outdoors under a structure with permeable frost cloth much like a typical covered kiwifruit canopy in order to simulate on-orchard conditions. However, early in the trial rain washed 'other' products off leaves prior to copper applications and it was decided that in order to fully assess the effects of product interactions and due to the exact timing of spray applications, it would be preferable to conduct experiments in a greenhouse. Plants were positioned in the lower, cooler end of the greenhouse with windbreak cloth adjacent to the plants on three sides (Figure 3.3). Data loggers were positioned inside the greenhouse so as to obtain temperature and relative humidity readings throughout the course of the trial.

Figure 3.3: Labelled, potted G3 plants positioned in lower end of greenhouse

Two copper formulations (Nordox® and Kocide®) and three 'other' spray formulations (Citrox BioAlexin™, Acadian and Kasumin™) were used in the trial. Citrox BioAlexin™ and Acadian were selected for the trial as in prior experiments they were found to strongly influence the release of Cu^{2+} when added to in copper solutions. Kasumin™ is now a widely used bactericide with the ability to control Streptomycin-resistant Psa. It was selected so as to obtain information on the potential influence on copper ion release and because an on-orchard trial could be conducted concurrently which would provide a useful comparison of results.

Spray products were applied at standard rates to leaves using a hand-held spray bottle with a fixed nozzle so as to maintain consistency in spray deposits (Figure 3.4). Copper sprays were applied with Du-Wett® as is standard practice. Water sensitive papers showed good coverage when two pumps of spray were applied to each leaf surface at a distance of 20cm and this application method was maintained throughout the trial.

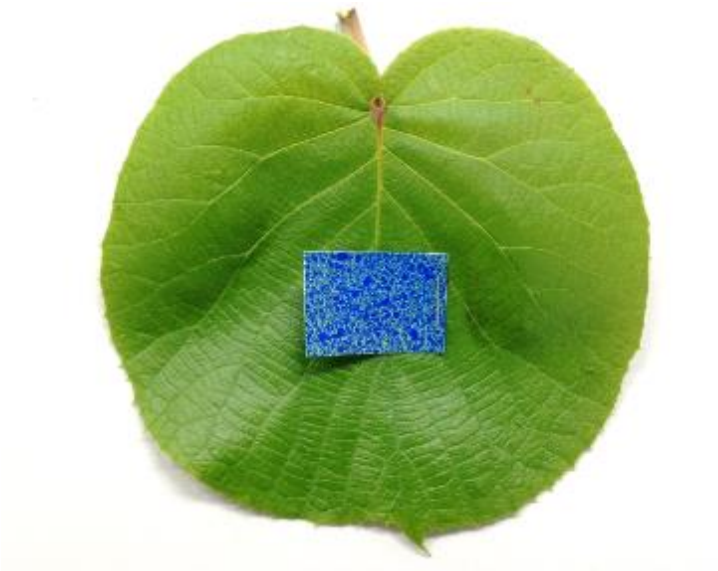

Figure 3.4: Water sensitive paper showing spray coverage using hand-held trigger sprayer at a distance of 20cm. Blue areas indicate spray droplet coverage.

The spray regime was designed so that 'other' spray products were applied at set time intervals prior to and after copper spray applications (Table 3.3). Control leaves were sprayed with copper and Du-Wett® only. Ten labelled leaves on an individual plant were sprayed for each treatment or control. Leaves in each treatment were paired with control leaves with similar properties in order to negate the effects of leaf age, health, morphology etc. on copper results.

Table 3.3: Spray regime for Year 2 greenhouse product interaction trial.

Spray formulation	Timing of application	Product rate L^{-1}
Nordox® control*	set intervals prior to or following 'other' spray application	0.375 g
Kocide® control*	set intervals prior to or following 'other' spray application	0.70 g
Acadian	-10, -6, -3, -2 -1 days prior; tank mixed; 1, 2, 3, 6, 10, 14, 21, 28 days post Nordox® or Kocide® applications	1.0 g
Citrox BioAlexin™	-10, -6, -3, -2 -1 days prior; tank mixed; 1, 2, 3, 6, 10 days post Nordox® or Kocide® applications	1.5 mL
Kasumin™	-10, -6, -3, -2 -1 days prior; tank mixed; 1, 2, 3, 6, 10 days post Nordox® or Kocide® applications	5 mL

* Du-Wett® was added at a standard rate of 0.25 mL L^{-1} to each copper formulation.

Spray regimes with different products were designed so as to limit the number of leaves sampled and tested in one day. This meant that products applied at certain time intervals either side of copper applications were not necessarily in the same time sequence. For example leaves sprayed -2 days and -1 day prior to copper may have been sprayed prior to the -6 and -10 day treatments. This meant that the treatment effects were determined according to measured differences between treatments and specific controls. In order to prevent spray drift a plastic tray was used to shield neighbouring leaves and prevent over-coverage (Figure 3.5). The time of spraying and sampling was recorded on each occasion so that results could be overlaid with temperature and %RH data.

Figure 3.5: Spray application with hand-held sprayer held at 20cm from leaf and using shield to prevent spray drift.

Greenhouse sub-trials

<u>Effect of leaf re-wetting on Cu^{2+} release</u>

Individual leaves were sprayed with either Nordox® or Kocide® as described previously and left for 24hrs. After 24hrs the same leaves were sprayed with deionized water, left to dry, and sampled. Measurements of Cu^{2+} release were undertaken as described previously.

<u>Sub-trial 2 – Effect of time of sampling</u>

Individual leaves were sprayed with either Nordox® or Kocide® as described previously and either sampled when dry (*status quo*), 24hrs or 48hrs after spraying a tank mix of Nordox® or Kocide® with Acadian. The magnitude of treatment effects as determined by differences between control leaves (copper only) and those sprayed with a tank mix on each of the three days were later compared. Measurements of Cu^{2+} release were undertaken as described previously.

<u>Sub-trial 3 – Effect of environmental conditions on Cu^{2+} release</u>

Two groups of potted G3 plants were positioned in either the greenhouse or immediately adjacent to the vine rows of a nearby G3 orchard block. Each group of plants were sprayed with Nordox® and Kocide® within an hour of each other. Temperature, humidity and rainfall were logged and dry leaves were sampled and measured over a 28 day period. Measurements of Cu^{2+} release were undertaken as described previously. Copper decay graphs constructed from the results of each group of plants were then compared.

In order to evaluate the persistence of total copper deposits on leaves after exposure to rain and other environmental variables, leaves were sampled at the end of the trial from the greenhouse and orchard. Total elemental copper still present on leaves was determined by ICP-MS at Brookside Laboratories. Eight leaves from each treatment were sampled and four composites of two leaves each were analysed together.

Orchard trial – Kasumin™

Trial setup and spray applications

Two orchards, one with G3 and one with the Hayward variety vines were used to determine the potential effects of Kasumin™ on copper ion release. A concurrent trial was being undertaken to provide information on Cu^{2+} release over time on orchard leaves subject to standard spray practice and exposed to typical environmental conditions. As Kasumin™ was to be applied as a tank mix with Kocide® this provided an opportunity to obtain results on spray interactions and results could be compared with those obtained in the greenhouse study.

Selected bays within each orchard were cordoned off with flagging tape to delineate treatment (Kocide® + Kasumin™) and control (Kocide®) leaf spraying/sampling zones (Figure 3.6). A buffer zone of one bay either side of the control bays was included to prevent spray drift between control and treatment areas.

A number of water-sensitive papers were affixed to selected upper and lower surfaces of leaves throughout each orchard to provide an indication of spray coverage. Data loggers were installed at each orchard so as to obtain temperature and humidity readings throughout the trial.

Figure 3.6: G3 orchard trial setup showing canopy cover in copper-only bays

The G3 orchard was sprayed with Kocide® on 6 October with an industry standard, axial fan, airblast sprayer (Atom) according to GroPlus standard practice. The copper spray had been made up in the atom tank approximately half an hour prior and transported to the orchard. Once Kocide® had been sprayed to the selected control bays, Kasumin™ was added to the tank at the recommended label rate and the rest of the orchard was sprayed with this product combination.

The Hayward orchard was sprayed on 20 October according to the procedures outlined above. However, on this occasion the copper was made up in the tank immediately prior to spraying. Leaves were sampled when dry and measurements of Cu^{2+} release were undertaken as described previously.

Orchard sub-trial – Citrox BioAlexin

Eight days after copper and Kasumin™ were sprayed on the Hayward canopy, Citrox BioAlexin™ was applied at the recommended label rate to twenty leaves in the same manner as the greenhouse trial. This was carried out in order to determine if copper ion release from total copper deposits on leaves could be induced. Leaves were sampled when dry and tested along with control leaves sampled within close proximity to and with similar characteristics to the treated leaves. Results from this sub-trial could also then be compared with results from the greenhouse trial using the same products.

Sampling and analysis

For the greenhouse trial eight leaves from each treatment and control plant were sampled into sealable bags once spray deposits had dried on leaf surfaces. For the orchard trials leaves from each of three positions on seven shoots (base, middle and tip) were sampled (21 leaves per treatment and control). Leaves were immediately transported to the laboratory and analyses were carried out as described previously in which calibration standards of known pCu^{2+} values were used to obtain Cu^{2+} concentration values from mV readings. The pH of wash solutions without the addition of ISA were measured at the time of pCu^{2+} measurements. Leaf areas were measured using a Burle TC1005 series camera connected to a Delta-T Devices Ltd MK2 area management system. Prior correlation analyses showed that leaf area results were highly comparable to Image J analyses.

Statistical analysis

Differences between means of treatments and controls were analysed for significance with Microsoft Excel t-tests assuming unequal variances using Microsoft Excel.

4. Results

Year 1

Rapid screening laboratory trial

The concentration of Cu^{2+} in solution varied greatly depending on the copper formulation, with the highest concentration in Nordox® solutions and the lowest in Coptyzin. When mixed with copper spray products, a number of 'other' spray products influenced the availability of Cu^{2+} in solution. The pH of the solution also varied depending on the product combination (Figures 4.1 – 4.3).

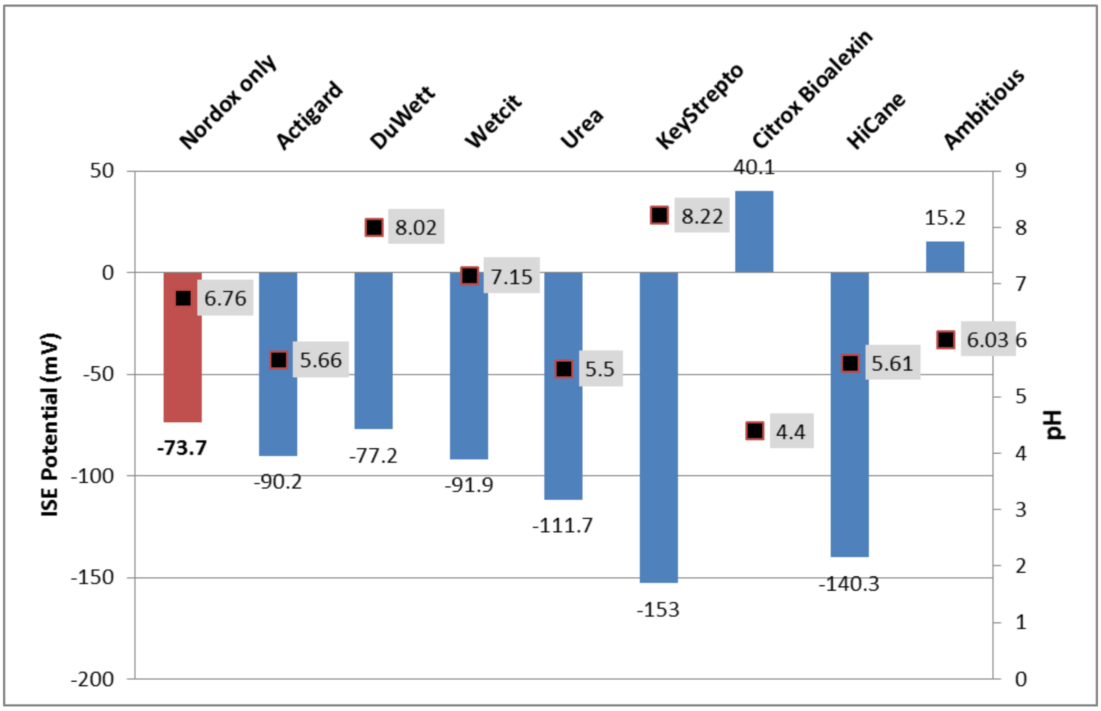

Figure 4.1: Effect of 'other' products on Cu^{2+} release in Nordox® solutions. Numbers below or above bars = potential where a lower number corresponds to fewer Cu^{2+} ions. Black & grey squares = pH. Readings taken in solutions with 10% v/v 5M KNO_3 ionic strength adjustor.

KeyStrepto™, Hi-Cane®, Acadian and foliar urea all greatly decreased the availability of Cu^{2+} in solutions of Nordox®. Conversely, Citrox BioAlexin™ and Ambitious™ both increased the availability of Cu^{2+}. Similarly with Kocide® Acadian decreased the availability of Cu^{2+} in solution and with Citrox BioAlexin™ there was a significant increase. However, the findings for the other products in Kocide® solutions were not consistent with Nordox® mixtures. Actigard™ and Du-Wett® both greatly decreased the availability of Cu^{2+} when mixed with Kocide®, whereas Hi-Cane® increased Cu^{2+} availability.

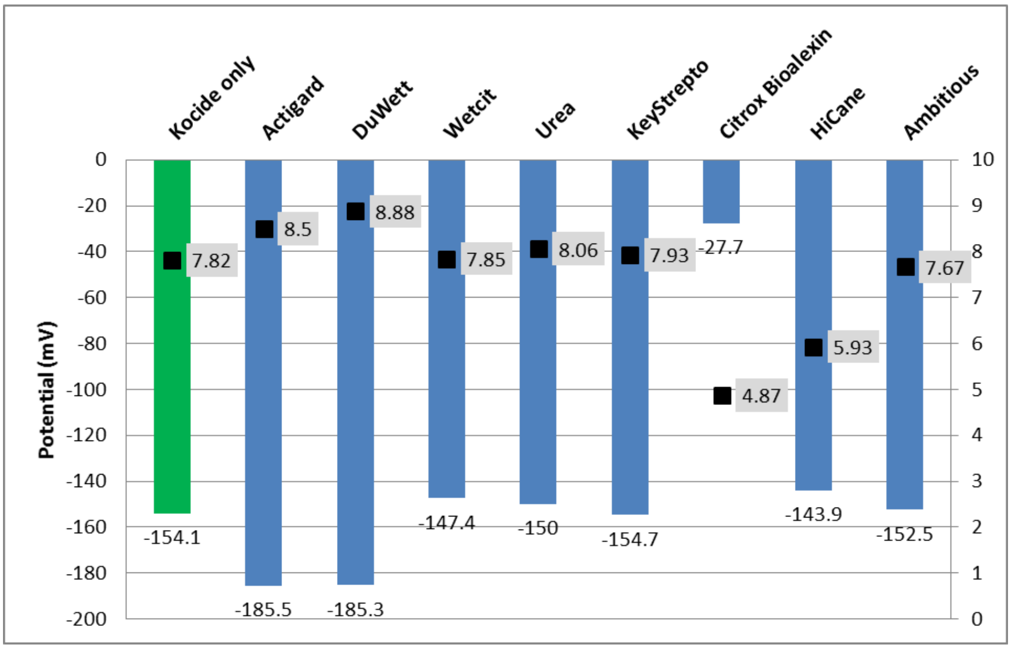

Figure 4.2: Effect of 'other' products on Cu^{2+} release in Kocide® solutions. Numbers below or above bars = potential where a lower number corresponds to fewer Cu^{2+} ions. Black & grey squares = pH. Readings taken in solutions with 10% v/v 5M KNO_3 ionic strength adjustor.

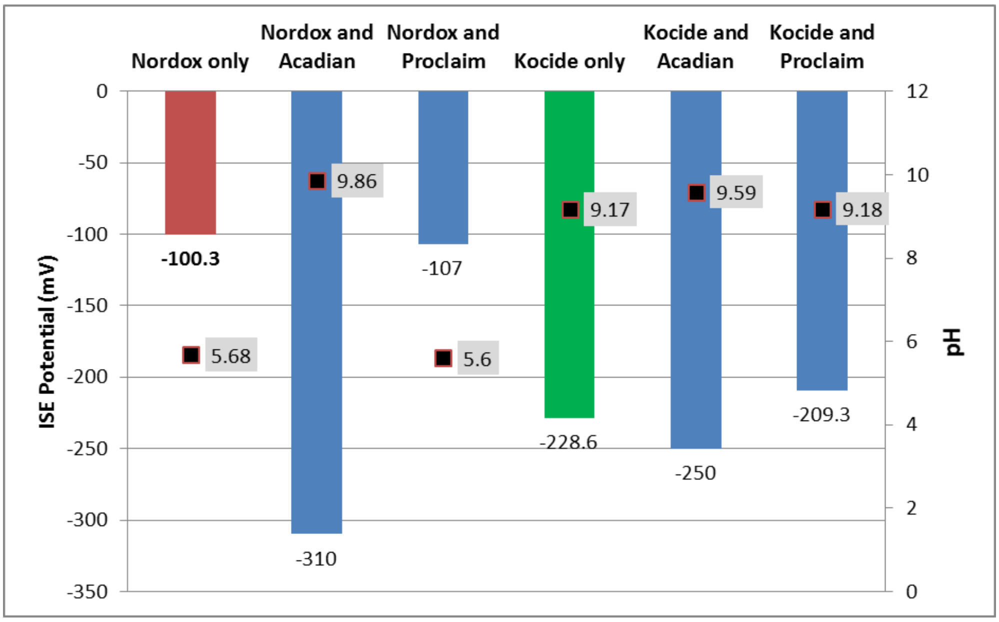

Figure 4.3: Effect of 'other' products on Cu^{2+} release in Nordox® and Kocide® solutions. Numbers below or above bars = potential where a lower number corresponds to fewer Cu^{2+} ions. Black & grey squares = pH. Readings taken in solutions with 10% v/v 1M KNO_3 ionic strength adjustor.

In solution with Coptyzin, Du-Wett®, WETCIT®, Actigard™, foliar urea, KeyStrepto™, Ambitious™, Hi-Cane® and Acadian all had the effect of reducing the concentration of Cu^{2+} in solution (Figure 4.4). As with the Nordox® and Kocide® mixtures, Citrox BioAlexin™ also greatly increased the concentration of Cu^{2+} in spray solution. However, unlike Nordox® and Kocide® mixtures, the pH of Coptyzin solutions remained largely unaffected by other products. The pH of the Coptyzin solution with Citrox BioAlexin™ was in fact slightly higher than that of Coptyzin alone.

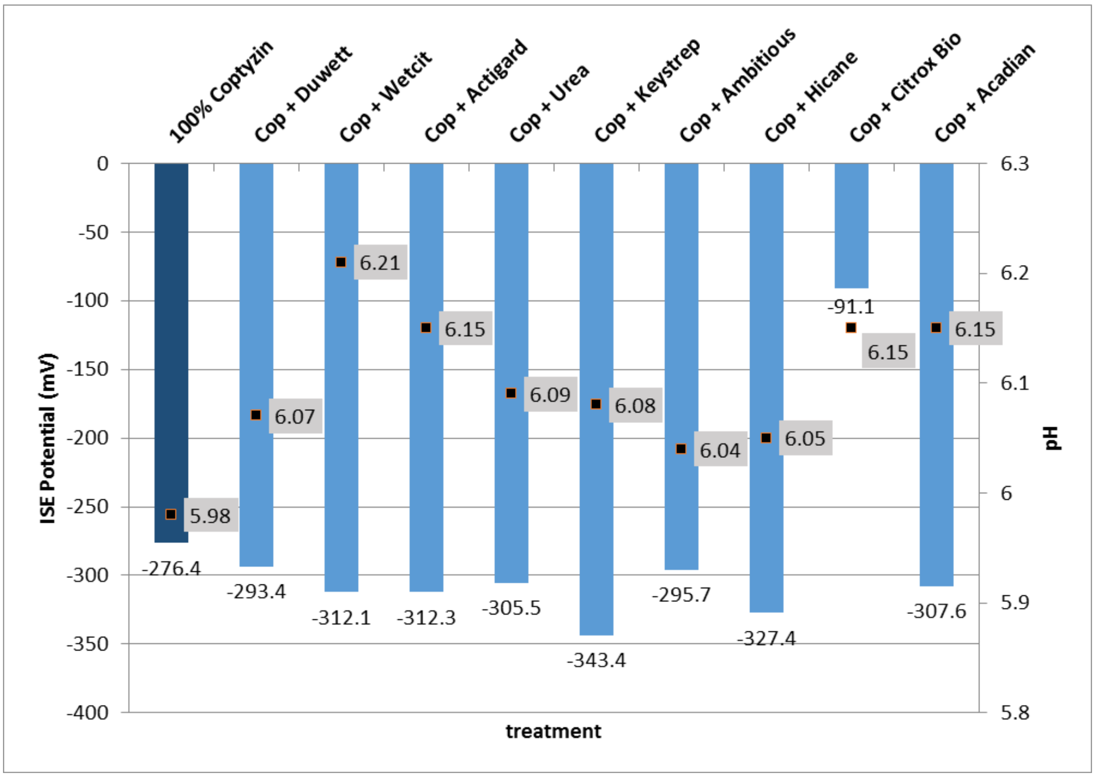

Figure 4.4: Effect of 'other' products on Cu^{2+} release in Coptyzin solutions. Numbers below or above bars = potential where a lower number corresponds to fewer Cu^{2+} ions. Black & grey squares = pH.

Differences between mV and pH readings for Nordox and Kocide only solutions (Figure 4.1, 4.2 and 4.3) were due to the addition of either 1M or 5M KNO_3 ISA, with the higher concentration having the effect of a larger reduction in pH and associated increase in Cu^{2+}.

Potted plant greenhouse trial

On the 20 March at the time of sampling, leaf surface temperatures averaged 12.5°C and on the 1 April temperatures averaged 17.5°C.

There was no significant reduction in Cu^{2+} release when Du-Wett® was used in combination with Nordox®. The Cu^{2+} recovered from the surfaces of leaves when treated with Acadian 24hrs prior to a Nordox®-Du-Wett® spray was significantly less (p = 0.001) than that for Nordox®-Du-Wett® only treated leaves (Figure 4.5). Interestingly the same results were not observed for leaves that had an Acadian application as a tank mix with or 24hrs after a Nordox®-Du-Wett® application. The application of a mixture of Actigard™ and Nordox®-Du-Wett® resulted in a significant increase in Cu^{2+} release on leaves.

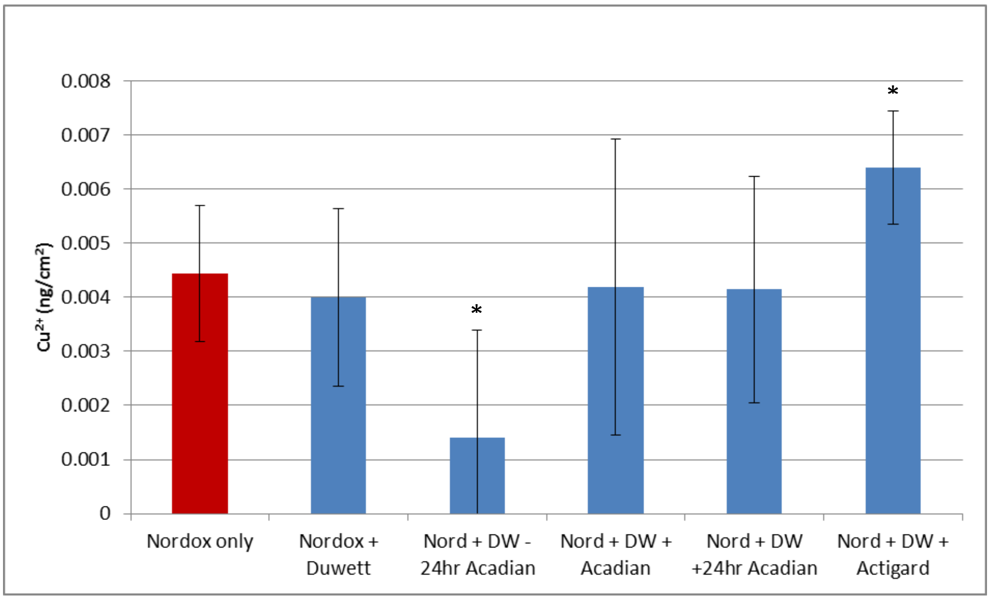

Figure 4.5: Cu^{2+} concentration in leaf washings - products sprayed on G3 leaves 17-19 March (n = 8). Error bars denote ± SD. * Significant at p < 0.05.

The release of Cu^{2+} on leaves was significantly greater (p = 0.05) when plants were sprayed with Citrox BioAlexin™ 24 hours prior to and 24 hours after a copper application (Figure 4.6). The application of KeyStrepto™ 24hrs prior to a copper application significantly reduced the number of available Cu^{2+} (p = 0.05). However, as with Acadian, the effect was not the same when the product was applied 24 hours after a copper application.

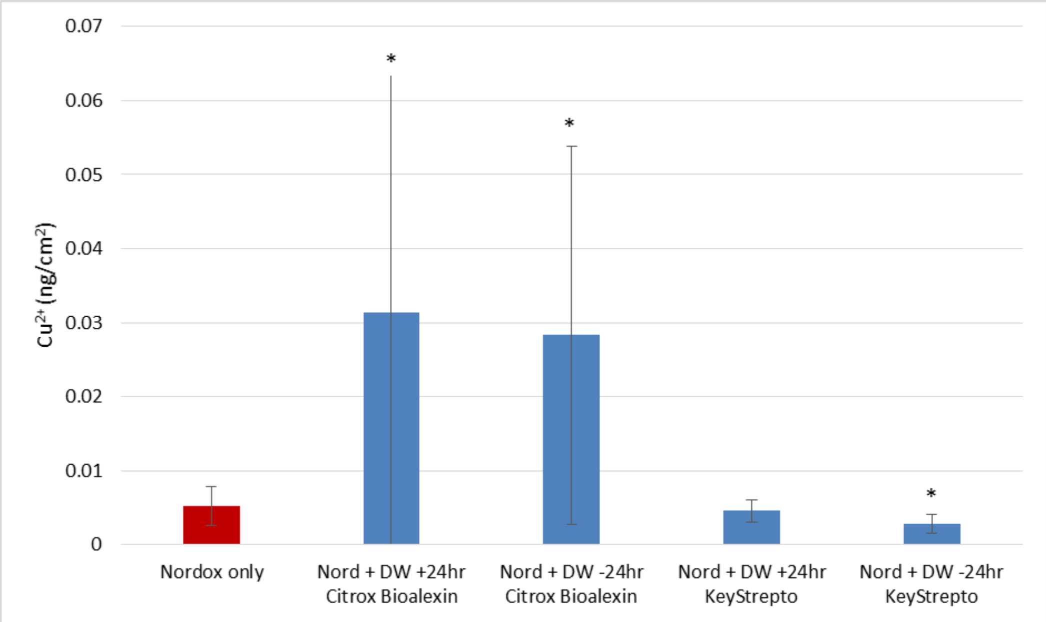

Figure 4.6: Cu^{2+} concentration in leaf washings - products sprayed on G3 leaves 30 March – 1 April. Error bars denote ± SD. * Significant at p <0.05

An addition of Du-Wett® to a Nordox® copper spray mix resulted in a significant increase in the leaf surface pH of treated leaves ($p < 0.05$). The pH of leaves was also significantly higher for those sprayed with Acadian as a tank mix and 24 hours prior to a Nordox®-DuWett® application than leaves sprayed with Nordox®-DuWett® only (

Table 4.1, Figure 4.7).

Table 4.1: Cu^{2+} and pH values for leaf wash solutions and leaf surfaces.

Product combination	mean Cu^{2+} (ng/cm^2)	mean leaf pH	mean solution pH
Nordox® only	0.0044	6.05	-
Nordox® + Du-Wett®	0.0040	6.13	-
Nordox® + Du-Wett® + Actigard™	0.0064	6.13	-
Nordox® + Du-Wett® + Acadian	0.0042	6.44	-
Nordox® + Du-Wett® -24hr Acadian	0.0014	6.50	-
Nordox® + Du-Wett® +24hr Acadian	0.0041	6.07	-
Nordox® + Du-Wett®	0.0052	-	6.12
Nord® + DW +24hr Citrox BioAlexin™	0.0313	-	5.99
Nord® + DW -24hr Citrox BioAlexin™	0.0283	-	6.08

Nord® + DW +24hr KeyStrepto™	0.0045	-	6.27
Nord® + DW -24hr KeyStrepto™	0.0028	-	6.25

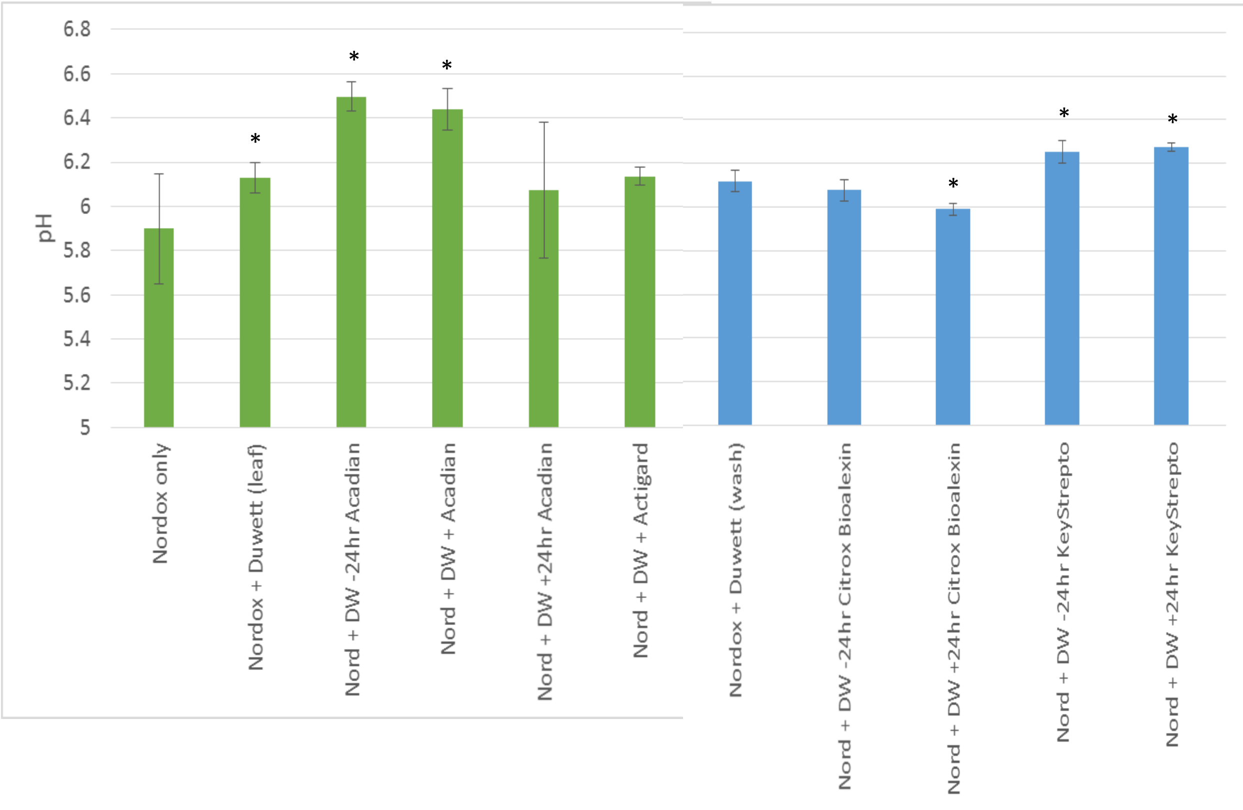

Figure 4.7: Leaf pH measurements for leaf surfaces (green) and leaf wash solutions (blue) corresponding to different spray treatments. Significant differences ($p < 0.05$) between treatments and controls are indicated with an asterisk.

The wash solution pH was within a very similar range as the leaf surface pH for leaves sprayed with Nordox® only (Figure 4.7). The wash solution pH for leaves sprayed with KeyStrepto™ before and after Nordox®-Du-Wett® applications were significantly higher than leaves sprayed with Nordox®-Du-Wett® only. The pH of wash solutions from leaves treated with Citrox BioAlexin™ 24 hours after Nordox®-Du-Wett® applications were significantly lower than control solutions.

The assessment of symptoms of phytotoxicity over the 12-16 day duration of this trial were confounded by thrip damage and natural senescence. However, there were no obvious signs of copper phytotoxicity, with the exception of one very young leaf that had been sprayed with Nordox® only.

Year 2

Potted plant greenhouse trial

Significant effects were observed between treatments and controls for all products. However, in a number of cases the magnitude of the effect depended on the timing of application of these products in relation to copper applications (Figure 4.8 & Figure 4.10). Whether or not the application of each product led to an increase or decrease in Cu^{2+} also depended on the timing of application. There was some variation in ambient temperature and humidity throughout the trial (Appendix B). Although, the magnitude of the effect on Cu^{2+} release for each product was always relative to control leaves sampled and measured at the same time.

Citrox BioAlexin™

Contrary to the results from the greenhouse study carried out in Year 1, Citrox BioAlexin™ applied 1 day prior to and after copper only had a negligible positive effect on Cu^{2+} release (Figure 4.8 & Table 4.2). When this product was applied between 2 and 10 days prior to Kocide®, fewer Cu^{2+} were released into solution. However, only the -3 and -6 day treatment results were significant ($p < 0.05$). When Citrox BioAlexin™ was applied to leaves 6, 10, 21 and 28 days after Kocide®, the amount of Cu^{2+} released into solution was significantly more than for control leaves. Although interestingly the observed increase for leaves sprayed 14 days post copper was smaller and not significant. Significant differences ranged from + 185% to + 236%.

When Citrox BioAlexin™ was applied prior to and up to 3 days post Nordox® applications, the results were mostly inconsistent and insignificant. As with the Kocide® interactions, significant increases in Cu^{2+} were observed when leaves were sprayed 10 to 21 days after Nordox®. The very high percentage increase in Cu^{2+} shown for the +10 day treatment (+ 674%) may be partly attributed to one very high reading, although exclusion from the dataset was not deemed necessary after conducting outlier analysis.

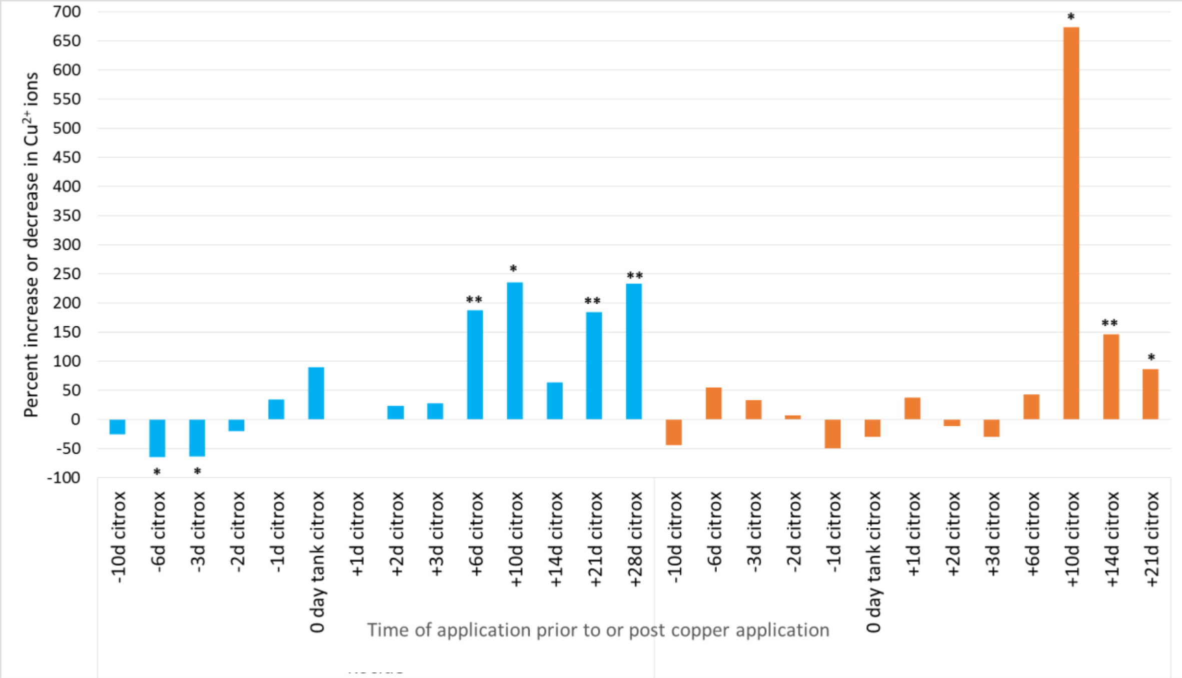

Figure 4.8: Percentage increase or decrease in Cu^{2+} concentration on leaves treated with Citrox BioAlexin™ as compared with concentrations on control leaves. Blue = Kocide®, orange = Nordox®. Significant differences between means are indicated where * = $p < 0.05$, ** = $p < 0.01$.

Table 4.2: Concentration of Cu^{2+} in leaf wash samples for leaves treated with Citrox BioAlexin™ and copper only controls. Significant differences between means are indicated where * = $p < 0.05$, ** = $p < 0.01$ (n = 8).

	Copper formulation					
	Nordox®			Kocide®		
	Mean Cu^{2+} on leaf surfaces (ng $cm^{2\,-1}$) ± SD					
Time of product app. (days)	Treatment	Control	% diff.	Treatment	Control	% diff.
- 10	0.44 ± 0.26	0.78 ± 0.64	- 25	0.85 ± 0.07	1.14 ± 0.38	- 25
- 6	0.16 ± 0.10	0.11 ± 0.04	+ 55	0.25 ± 0.11	0.72 ± 0.52	- 64 *
- 3	0.14 ± 0.08	0.11 ± 0.04	+ 33	0.26 ± 0.18	0.72 ± 0.52	- 63 *
- 2	0.60 ± 0.23	0.56 ± 0.35	+ 8	0.49 ± 0.31	0.61 ± 0.33	- 20
- 1	0.28 ± 0.14	0.56 ± 0.35	- 49	0.83 ± 0.44	0.61 ± 0.33	+ 35
tank mix	0.40 ± 0.14	0.56 ± 0.35	- 29	1.17 ± 0.67	0.61 ± 0.33	+ 90
+ 1	0.27 ± 0.11	0.20 ± 0.12	+ 38	0.33 ± 0.24	0.32 ± 0.24	+ 1
+ 2	0.29 ± 0.28	0.32 ± 0.13	- 11	0.59 ± 0.16	0.48 ± 0.27	+ 24
+ 3	0.26 ± 0.14	0.36 ± 0.22	- 29	0.37 ± 0.38	0.28 ± 0.38	+ 28
+ 6	0.50 ± 0.24	0.35 ± 0.13	+ 43	0.65 ± 0.33	0.23 ± 0.18	+ 188 **
+ 10	0.43 ± 0.36	0.06 ± 0.07	+ 674 *	0.29 ± 0.18	0.08 ± 0.05	+ 236 *
+ 14	0.32 ± 0.13	0.13 ± 0.08	+ 147 **	0.42 ± 0.21	0.25 ± 0.18	+ 64
+ 21	0.38 ± 0.13	0.21 ± 0.11	+ 86 *	0.33 ± 0.16	0.11 ± 0.16	+ 185 **
+ 28	-	-	-	0.22 ± 0.07	0.07 ± 0.05	+ 233 **

The pH of leaf wash solutions from treatment leaves was generally but not consistently lower than solutions from control leaves (Table 4.3). For nine treatments where differences were significant, the mean pH of one set of solutions (+ 2 days) was actually higher than those of the controls.

Table 4.3: pH of leaf wash solutions for leaves treated with Citrox BioAlexin™ and copper only controls. Significant differences between means are indicated where * = *p* < 0.05, ** = *p* < 0.01 (n = 8).

	Copper formulation			
	Nordox®		Kocide®	
Time of product app. (days)	Mean pH of leaf wash solutions			
	Treatment	Control	Treatment	Control
- 10	5.87	5.78	6.14	6.12
- 6	5.69	5.70	6.01	5.93
- 3	5.59*	5.70	5.86	5.93
- 2	5.81	5.95	6.00	5.98
- 1	5.85	5.95	5.56**	5.98
tank mix	5.53**	5.95	5.66**	5.98
+ 1	5.76	5.75	6.00	5.97
+ 2	5.88*	5.75	5.76**	5.90
+ 3	5.53*	5.65	5.81	5.74
+ 6	5.64	5.58	5.65	5.71
+ 10	5.76*	5.99	5.80	5.78
+ 14	5.81*	6.03	5.81	5.91
+ 21	5.73	5.76	5.62	5.73
+ 28	N/A	N/A	5.76	5.75

<u>Acadian</u>

The seaweed product Acadian consistently and negatively influenced the release of Cu^{2+} on leaves when applied up to 10 days prior to, as a tank mix, and 10 days after both copper applications (Figure 4.9 & Table 4.4). Significant reductions of up to 95% were observed for both product combinations with the exception of five timed applications. However, the measured differences between these control and treatment means were close to being significant.

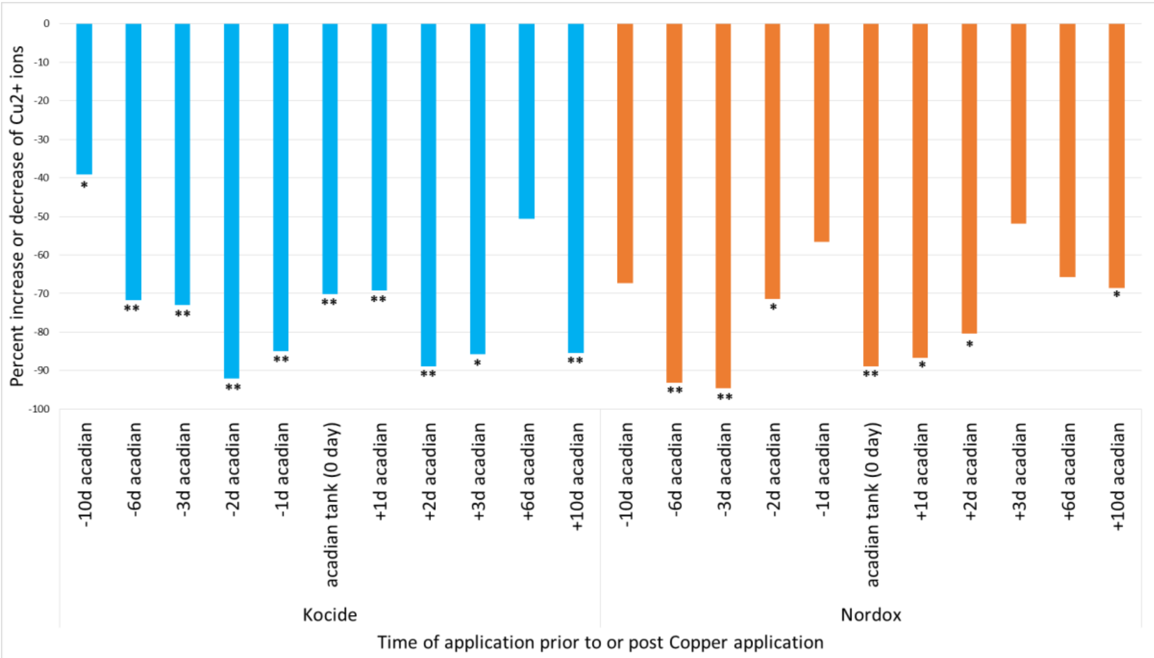

Figure 4.9: Percentage increase or decrease in Cu^{2+} concentration on leaves treated with Acadian as compared with concentrations on control leaves. Blue = Kocide®, orange = Nordox®. Significant differences between means are indicated where * = *p* < 0.05, ** *p* = < 0.01.

Table 4.4: Concentration of Cu^{2+} in leaf wash samples for leaves treated with Acadian and copper only controls. Significant differences between means are indicated where * = *p* < 0.05, ** = *p* < 0.01 (n = 8).

Time of product app. (days)	Copper formulation					
	Nordox®			Kocide®		
	Mean Cu^{2+} on leaf surfaces (ng $cm^{2\,-1}$) ± SD					
	Treatment	Control	% diff.	Treatment	Control	% diff.
- 10	0.26 ± 0.18	0.78 ± 0.64	- 67	0.69 ± 0.44	1.14 ± 0.38	- 39 *
- 6	0.07 ± 0.03	0.96 ± 0.29	- 93**	0.56 ± 0.29	1.97 ± 0.48	- 72 **
- 3	0.05 ± 0.03	0.96 ± 0.29	- 95 **	0.53 ± 0.24	1.97 ± 0.48	- 73 **
- 2	0.13 ± 0.08	0.45 ± 0.29	- 71 *	0.15 ± 0.08	1.86 ± 0.64	- 92 **
- 1	0.19 ± 0.14	0.45 ± 0.29	- 57	0.28 ± 0.16	1.86 ± 0.64	- 85 **
tank mix	0.15 ± 0.08	1.35 ± 0.82	- 89 **	0.65 ± 0.31	2.19 ± 0.95	- 70 **
+ 1	0.04 ± 0.02	0.30 ± 0.23	- 87 *	0.06 ± 0.05	0.20 ± 0.10	- 69 **
+ 2	0.03 ± 0.03	0.14 ± 0.09	- 80 *	0.02 ± 0.01	0.16 ± 0.10	- 89 **
+ 3	0.07 ± 0.03	0.15 ± 0.11	- 52	0.02 ± 0.01	0.16 ± 0.12	- 86 *
+ 6	0.03 ± 0.02	0.10 ± 0.08	- 66	0.06 ± 0.03	0.11 ± 0.10	- 51
+ 10	0.03 ± 0.02	0.11 ± 0.06	- 69 *	0.03 ± 0.02	0.22 ± 0.11	- 86 **

Kasumin™

As with Citrox BioAlexin™, the degree of difference between Kasumin™ treatments and controls were inconsistent and highly variable (Figure 4.10 & Table 4.5).

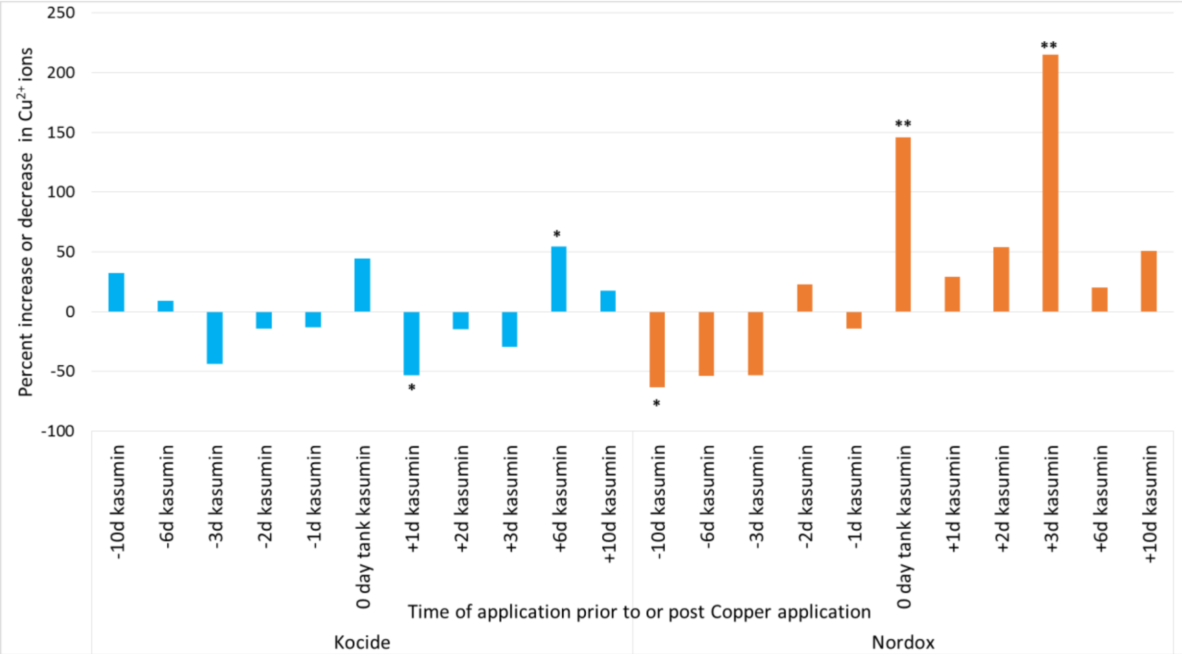

Figure 4.10: Percentage increase or decrease in Cu^{2+} concentration on leaves treated with Kasumin™ as compared with concentrations on control leaves. Blue = Kocide®, orange = Nordox®. Significant differences between means are indicated where * = *p* < 0.05, ** *p* = < 0.01.

The effect of this product on Cu^{2+} release, which may be either positive or negative appears to relate to the timing of application. However, only five measured differences were significant. The tank mixing of Kasumin™ with either copper formulation led to an increase in Cu^{2+} release on leaves, with the effect on Nordox® highly significant (+ 146%, $p = 0.003$).

Table 4.5: Concentration of Cu^{2+} in leaf wash samples for leaves treated with Kasumin™ and copper only controls. Significant differences between means are indicated where * = *p* < 0.05, ** = *p* < 0.01 (n = 8).

	Copper formulation					
	Nordox®			Kocide®		
Time of product app. (days)	Mean Cu^{2+} on leaf surfaces (ng cm$^{2\ -1}$) ± SD					
	Treatment	Control	% diff.	Treatment	Control	% diff.
- 10	0.11 ± 0.05	0.31 ± 0.22	- 64 *	0.45 ± 0.24	0.34 ± 0.19	+ 32
- 6	0.14 ± 0.08	0.31 ± 0.22	- 54	0.37 ± 0.19	0.34 ± 0.19	+ 9
- 3	0.15 ± 0.05	0.31 ± 0.22	- 53	0.19 ± 0.06	0.34 ± 0.19	- 44
- 2	0.96 ± 0.27	0.78 ± 0.67	+ 23	1.56 ± 0.53	1.82 ± 1.14	- 14
- 1	0.67 ± 0.14	0.78 ± 0.67	- 14	1.59 ± 0..41	1.82 ± 1.14	- 13
tank mix	1.92 ± 0.62	0.78 ± 0.67	+ 146 **	2.64 ± 0.58	1.82 ± 1.14	+ 45
+ 1	0.57 ± 0.14	0.44 ± 0.22	+ 29	0.20 ± 0.07	0.42 ± 0.19	- 53 *
+ 2	0.58 ± 0.38	0.38 ± 0.14	+ 54	0.30 ± 0.14	0.36 ± 0.19	- 15
+ 3	0.66 ± 0.34	0.21 ± 0.10	+ 215 **	0.30 ± 0.18	0.42 ± 0.25	- 30
+ 6	0.98 ± 0.49	0.82 ± 0.36	+ 20	0.70 ± 0.22	0.22 ± 0.45	+ 54 *
+ 10	0.30 ± 0.19	0.20 ± 0.11	+ 51	0.28 ± 0.15	0.23 ± 0.16	+ 18

Phytotoxicity

Very few signs of phytotoxicity were noted throughout the greenhouse trial. However, some noticeable areas of darkening around the leaf margins were noted on two occasions immediately after Kocide® applications. Plants sprayed 10 days prior to Kocide® with Acadian and Citrox BioAlexin™ showed the most noticeable symptoms (Figure 4.11, Appendix C). The temperature at the time of spraying was between 29 and 30°C (Appendix B) with high sunlight intensity. All of the eight Acadian-treated leaves showed significant signs of phytotoxicity after Kocide® applications and seven out of eight leaves treated with Citrox BioAlexin™ displayed signs of phytotoxicity to a lesser degree. Three out of eight control leaves also displayed the same symptoms to approximately the same degree as the Citrox BioAlexin™ treated leaves (Figure 4.12, Appendix C).

Figure 4.11: Signs of phytotoxicity on leaves sprayed with Kocide® 10 days after previous applications of Acadian (left) and Citrox BioAlexin™ (right).

Figure 4.12: Signs of phytotoxicity on a leaf sprayed with Kocide® only.

Symptoms of phytotoxicity were also noticed on seven out of eight leaves after treatment with a tank mixture of Kocide® and Acadian. Two out of eight control leaves sprayed with Kocide® only also displayed similar symptoms (Figure 4.13). The temperature at the time of spraying and sampling was between 21 and 23°C and symptoms observed were less pronounced than those noted previously.

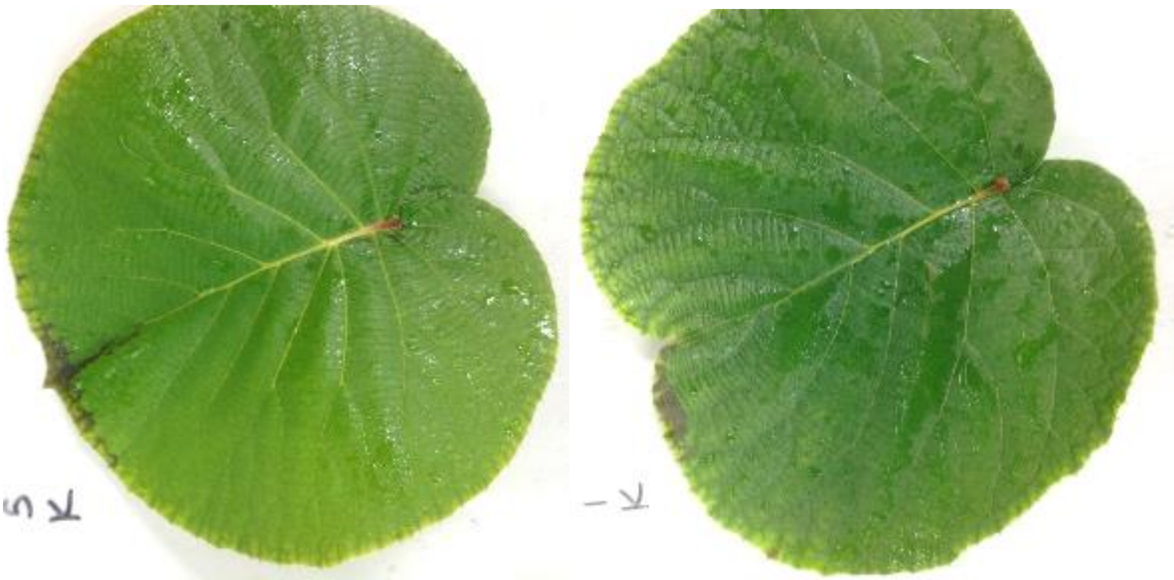

Figure 4.13: Signs of phytotoxicity on a leaf sprayed with a mixture of Kocide® and Acadian (left) and Kocide® only (right).

Phytotoxicity was not noted on leaves treated with Kocide® on any other occasion nor on any leaves treated with Nordox®.

Orchard trials

Kasumin™

The results of the orchard trials revealed that Kasumin™, when applied as a tank mix with Kocide®, had the effect of stimulating Cu^{2+} release on leaf surfaces on mature G3 and Hayward vines (Figure 4.14 and Figure 4.15).

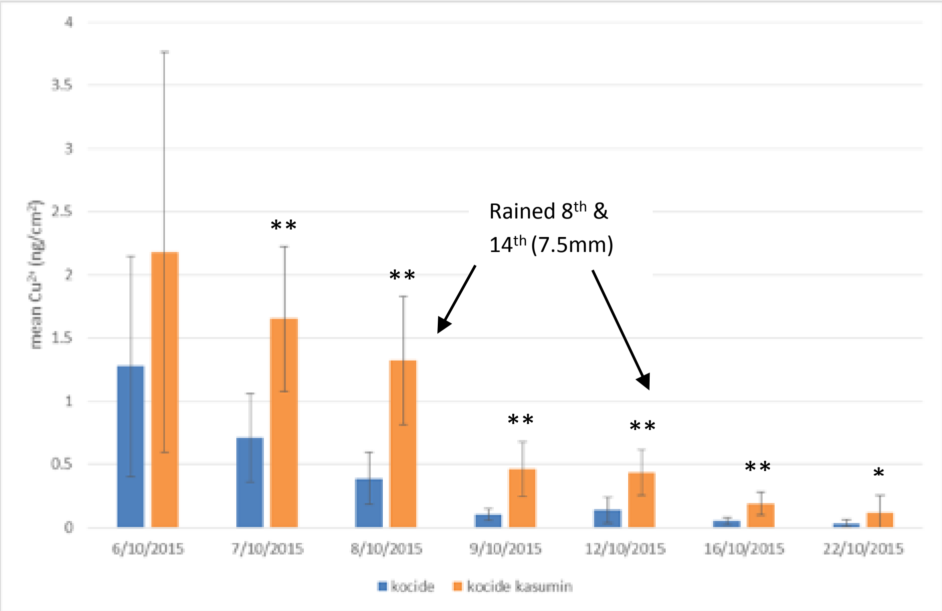

Figure 4.14: Mean Cu^{2+} release over time on leaf surfaces of G3 orchard vines sprayed with Kocide® or a Kocide®-Kasumin™ tank mix. Error bars denote ± SD. Significant differences between means are indicated where * = $p < 0.05$, ** = $p < 0.01$.

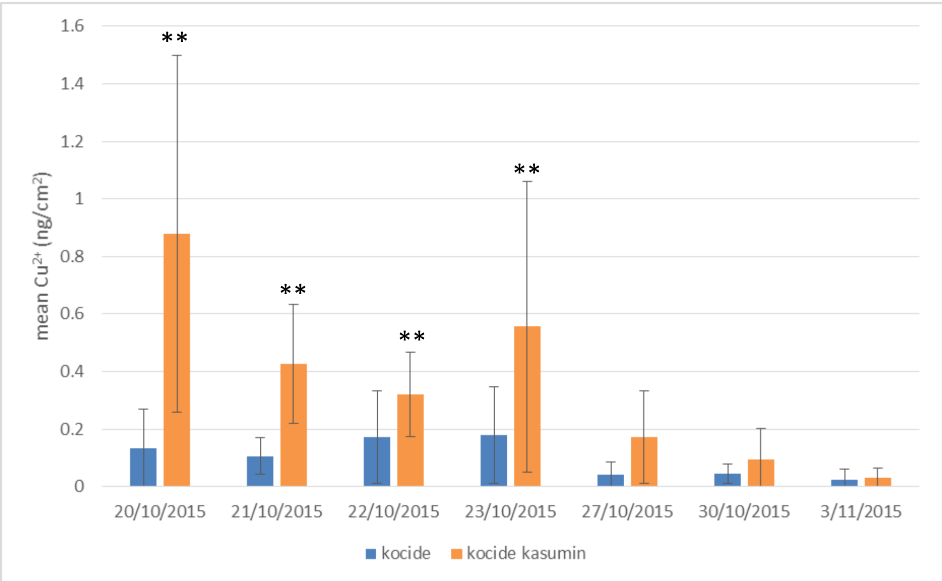

Figure 4.15: Mean Cu^{2+} release over time on leaf surfaces of Hayward orchard vines sprayed with Kocide® or a Kocide®-Kasumin™ tank mix. Error bars denote ± SD. Significant differences between means are indicated where ** = $p < 0.01$.

The G3 orchard received a total of 7.5mm of rain from 2 rainfall events on the 8th and 14th of March. This may have resulted in a loss of total copper deposits from which Cu^{2+} could be released. However, the effect of tank-mixing copper with Kasumin™ is apparent from the first day of sampling and these results concur with those from the greenhouse study.

There was a high degree of variability in initial total copper deposition throughout and between orchards as indicated by water sensitive papers (Appendix A). Measured Cu^{2+} readings were also highly variable within this trial and also when compared with readings obtained from the greenhouse trial. Therefore results have also been expressed in terms of Cu^{2+} relative to the expected total copper deposition as determined by ICP-MS analysis of adjacent leaves (Figure 4.16). This also allowed for a better comparison of spray interactions on the two different kiwifruit varieties.

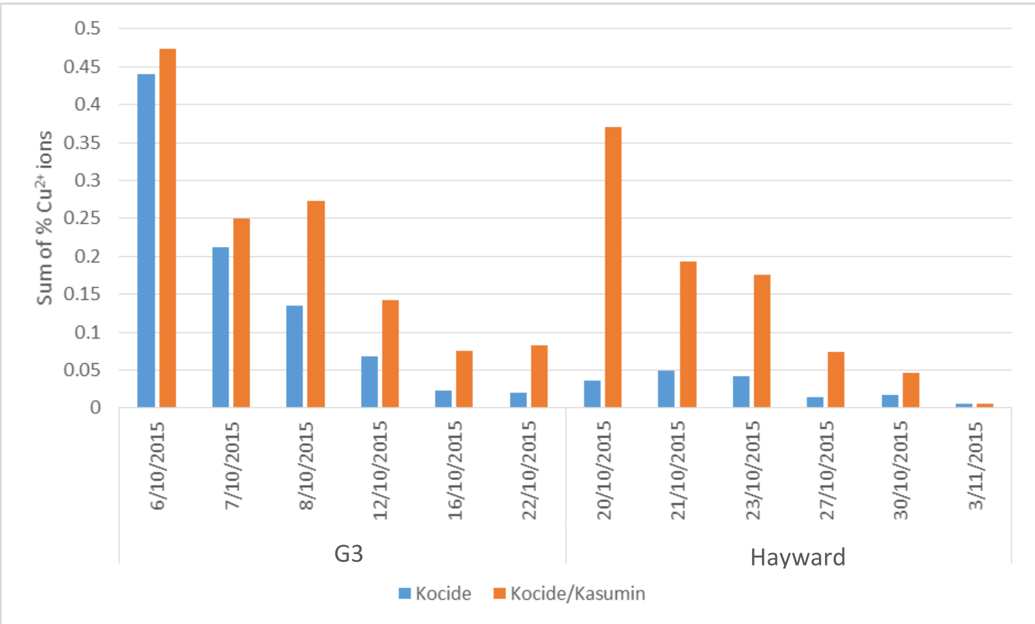

Figure 4.16: Concentration of Cu^{2+} expressed as a percentage of expected total elemental copper in Kocide® deposits on leaves of mature G3 and Hayward orchard vines.

Copper ion release on G3 and Hayward vine leaves treated with a Kocide®/Kasumin™ mix was consistently higher than on leaves treated with Kocide® only. Although, the measured difference between treatments and controls for Hayward leaves was much greater. As values expressed were calculated from mean values, standard errors and levels of significance could not be calculated.

Citrox BioAlexin™

Results of the sub-trial on copper reactivation clearly show that application of Citrox BioAlexin™ to leaves sprayed eight days prior with Kocide®, results in a significant increase in Cu^{2+} release on leaf surfaces (Figure 4.17). For many treated leaves the concentration of Cu^{2+} was almost twofold the concentration on control leaves.

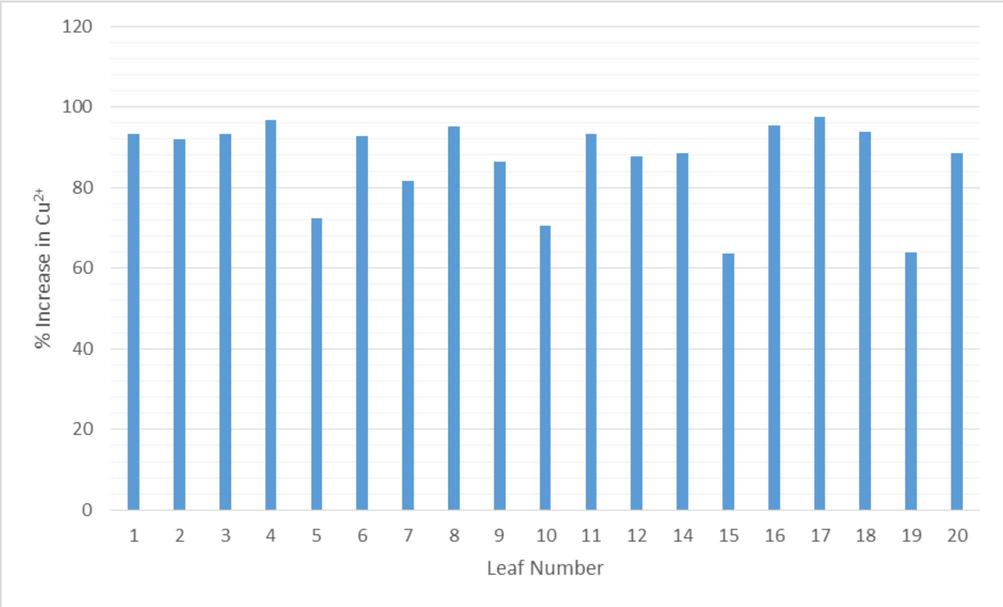

Figure 4.17: Percentage increase in Cu^{2+} concentration on leaves treated with Citrox BioAlexin™ eight days after Kocide® application as compared with concentrations on control leaves.

Greenhouse sub-trials

Effect of water spray on Cu^{2+} release

There were no significant differences between means of Cu^{2+} concentration on treated and control leaves for either copper formulation when deionized water was sprayed onto treated leaves 24hrs after copper applications (Figure 4.18). The concentration of Cu^{2+} in leaf wash solutions for Nordox® controls and water treatments were almost identical. Although, the mean concentration of Cu^{2+} on Kocide®-treated leaves re-wetted with water appeared to be higher than the controls, there was also a high degree of variability between replicates. These results suggest that differences between leaves treated with product combinations and controls are due to product interactions and not leaf re-wetting.

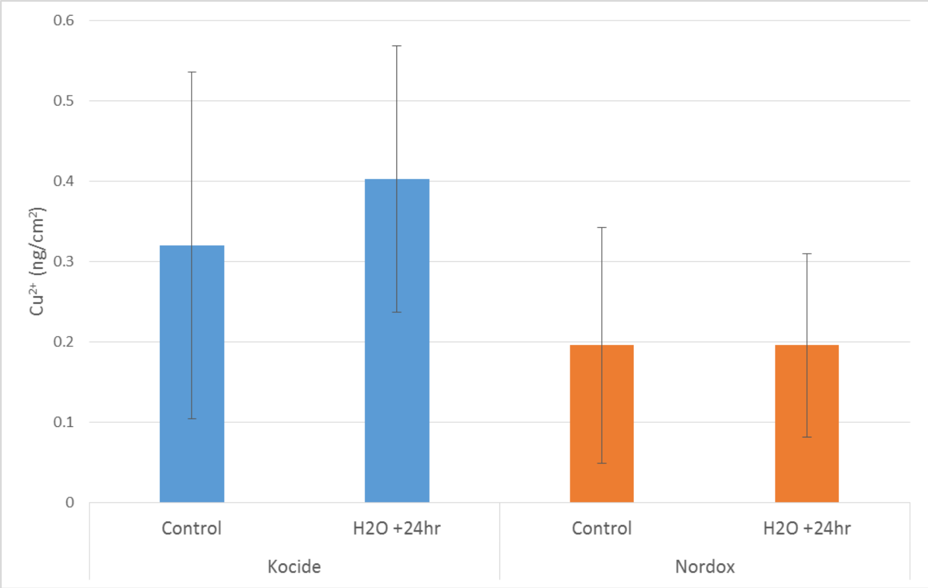

Figure 4.18: Comparison of Cu^{2+} concentration on leaves sprayed with deionized water 24hrs post copper application and control leaves (n = 15). Error bars denote ± SD.

Copper interactions - effect of time of sampling

The measured difference in Cu^{2+} concentration between control leaves and leaves treated with a copper + Acadian mix varied depending on the time at which leaves were sampled post-spray (Figure 4.19 & Table 4.6).

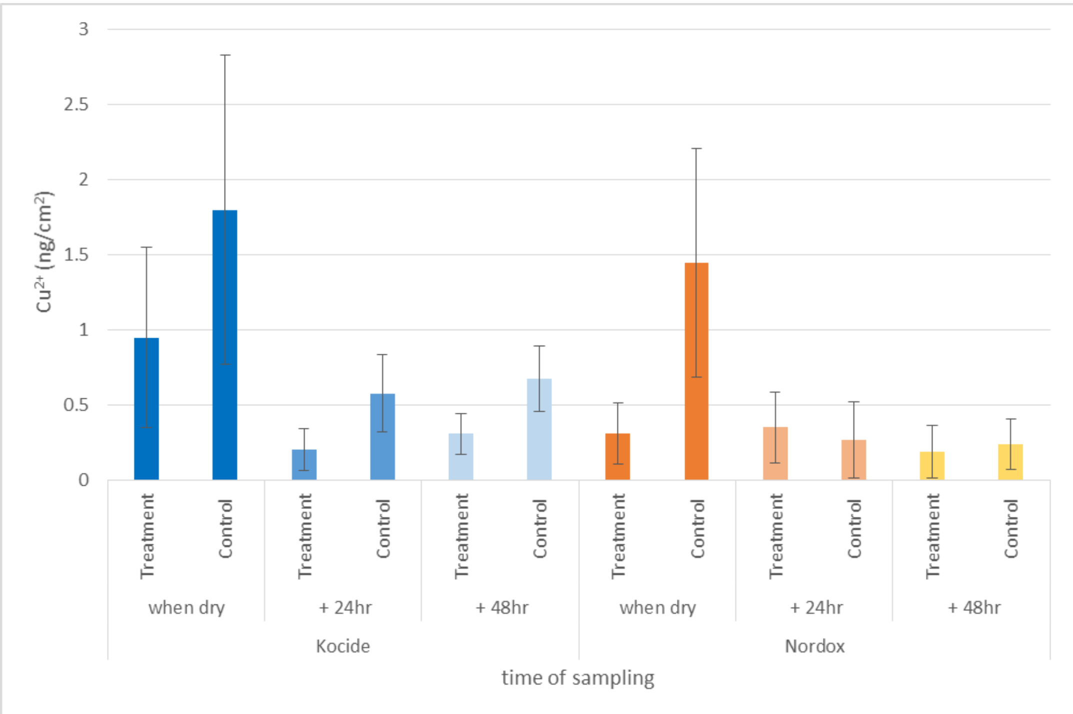

Figure 4.19: Effect of time of sampling on differences in mean Cu^{2+} concentration on leaves treated with a copper-Acadian mix and control leaves (n = 8). Error bars denote ± SD.

For Kocide®, the differences between treatments and controls on each date were similar (within 17%). Although for Nordox®, there was high variability and inconsistency in the degree of difference for the three sampling times. The percentage difference in the concentration of Cu^{2+} on treatment leaves varied from - 78.5% to + 30% (Table 4.6).

Table 4.6: Differences in Cu^{2+} concentration on leaves treated with a copper-Acadian mix and control leaves as a function of the time of sampling (n = 8).

	Kocide®			Nordox®		
	Cu^{2+} (ng cm^{-2}) ± SD			Cu^{2+} (ng cm^{-2}) ± SD		
Time of sampling	Treatment	Control	% diff.	Treatment	Control	% diff.
when dry	0.95 ± 0.60	1.80 ± 1.03	- 47.3	0.31 ± 0.20	1.45 ± 0.76	- 78.5
+ 24hr	0.21 ± 0.14	0.58 ± 0.26	-64.1	0.35 ± 0.23	0.27 ± 0.25	+ 30.0
+ 48hr	0.31 ± 0.14	0.67 ± 0.22	- 53.7	0.19 ± 0.17	0.24 ± 0.17	- 20.9

Effect of environmental conditions on Cu^{2+} release

There were 10 sampling occasions throughout this month-long trial. On the day of initial spray applications, the differences in Cu^{2+} release on leaves sprayed with both copper formulations were significantly different with higher concentrations measured from orchard leaves (Figure 4.20 & Table 4.7). Although, between days 15, 21 and 28, Cu^{2+} release on orchard leaves sprayed with Nordox® were significantly lower than greenhouse leaves. Significant differences were also measured for leaves sprayed with Kocide® on four occasions. However, concentrations on leaves sampled from the orchard were higher on days 1 and 2 and lower on days 15 and 28.

A general decrease in mean Cu^{2+} concentrations on leaves sampled from the orchard were observed over time, with the exception of a spike in Cu^{2+} on leaves sampled on day 9. This general trend is consistent with the results of the orchard study. Interestingly, after an initial decrease in Cu^{2+} over the first 4 days for greenhouse samples, the concentration of Cu^{2+} on leaves sprayed with either copper formulation then increased between day 4 and day 28.

Between 14 March (Day 0), when the trial plants were sprayed with copper, and 11 April (Day 28) when the last leaves were sampled, a total of 192mm of rain was recorded at the orchard from several rainfall events (Table 4.7).

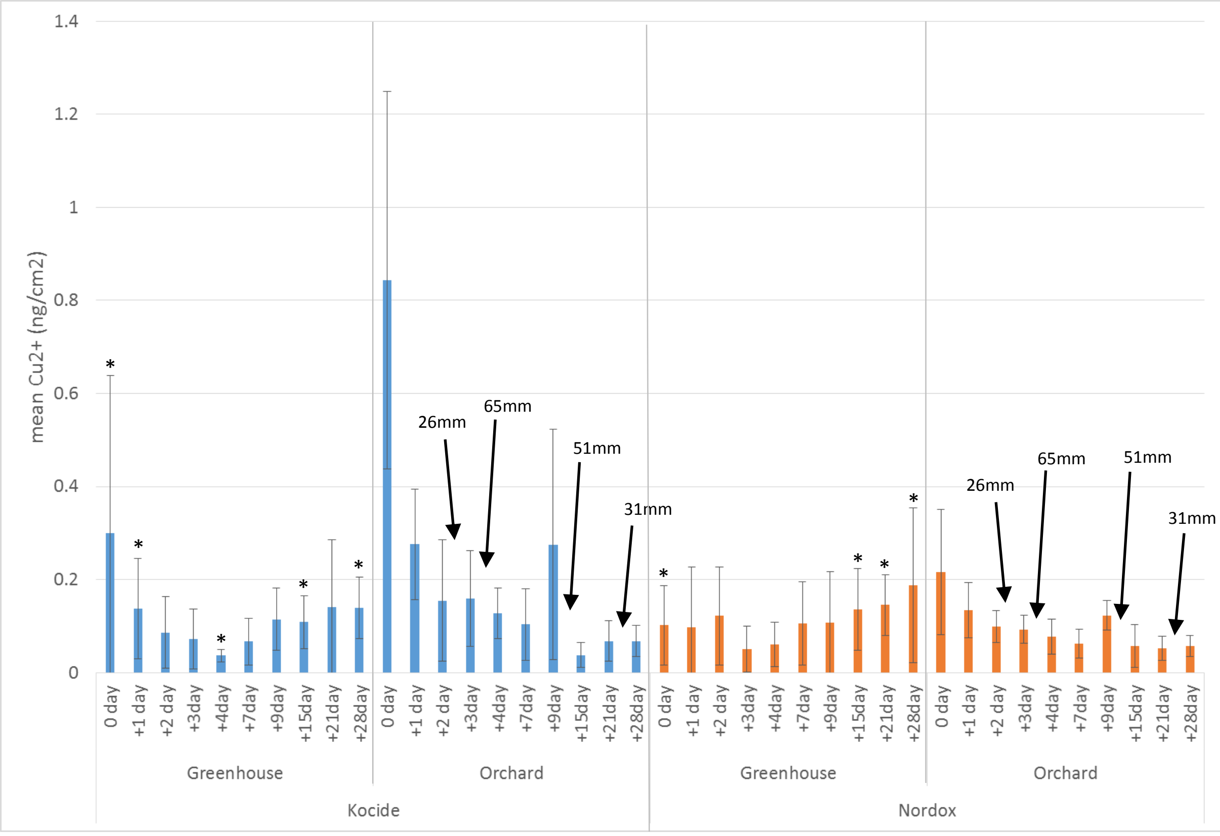

Figure 4.20: Comparison of Cu^{2+} concentration on leaves of potted plants positioned in a greenhouse and orchard over a 4 week period. Error bars denote ± SD. Significant differences between results from the greenhouse and orchard for each sampling time are indicated where * = *p* < 0.05 (n = 8). Significant rainfall events are indicated with arrows.

Table 4.7: Comparison of Cu^{2+} concentration on leaves of potted plants positioned on orchard and in a greenhouse (n = 8). Significant differences between means are indicated where * = *p* < 0.05.

			Kocide®			Nordox®		
			Cu^{2+} (ng cm^{-2}) ± SD			Cu^{2+} (ng cm^{-2}) ± SD		
time of sampling	Date	rainfall (mm)	greenhouse	orchard	% diff.	greenhouse	orchard	% diff.
Day 0	14/03/16	-	0.30 ± 0.34	0.84 ±	-181*	0.10 ± 0.09	0.22 ± 0.13	-111*
Day 1	15/03/16	-	0.14 ± 0.08	0.28 ±	-101*	0.10 ± 0.13	0.13 ± 0.06	-37
Day 2	16/03/16	4.3	0.09 ± 0.06	0.15 ±	-79	0.12 ± 0.10	0.10 ± 0.03	18
Day 3	17/03/16	26	0.07 ± 0.01	0.16 ±	-121	0.05 ± 0.05	0.09 ± 0.03	-84
Day 4	18/03/16	65	0.04 ± 0.05	0.13 ±	-246*	0.06 ± 0.05	0.08 ± 0.04	-26
Day 7	21/03/16	7.8	0.07 ± 0.07	0.10 ±	-54	0.11 ± 0.09	0.06 ± 0.03	41
Day 9	23/03/16	1	0.11 ± 0.06	0.28 ±	-140	0.11 ± 0.11	0.12 ± 0.03	-14
Day 15	29/03/16	51	0.11 ± 0.09	0.04 ±	65*	0.14 ± 0.09	0.06 ± 0.05	58*
Day 21	04/04/16	31	0.14 ± 0.16	0.07 ±	52	0.15 ± 0.07	0.05 ± 0.03	63*
Day 28	11/04/16	8	0.14 ± 0.07	0.07 ±	52*	0.19 ± 0.17	0.06 ± 0.02	70*

Throughout the duration of the trial temperatures within the greenhouse were on average 2°C warmer than the orchard. The temperature within the greenhouse ranged from 7.6°C to 38.6°C compared with orchard temperatures which ranged from 6.6°C to 29.1°C (Appendix D). On average, the percentage relative humidity (%RH) within the greenhouse was 6.5% lower than the orchard. Between 17 March (Day 3) and 25 March (Day 11), relative humidity at the orchard remained consistently above 70%, whereas in the greenhouse levels dropped below 50% during the afternoon. Differences in temperature and %RH at the time of leaf sampling on a number of occasions were also observed (Table 4.8). However, there does not seem to be a correlation between differences in temperature and %RH at the time of leaf sampling and differences in Cu^{2+} between orchard and greenhouse leaves. Neither was there a correlation between temperature and %RH and the anomalous Cu^{2+} readings on Day 9 for orchard samples.

Table 4.8: Temperature and relative humidity (%RH) readings at the time of leaf sampling.

		Temperature °C			%RH		
time of sampling	Date	greenhouse	orchard	% diff.	greenhouse	orchard	% diff.
Day 0	14/03/16	20.6	22.6	-2.0	79.2	64.5	14.7
Day 1	15/03/16	30.6	22.6	8.0	44.7	65.0	-20.3
Day 2	16/03/16	30.6	26.1	4.5	57.6	66.2	-8.6
Day 3	17/03/16	20.1	19.6	0.5	86.2	84.8	1.4
Day 4	18/03/16	21.1	25.6	-4.5	82.5	70.9	11.4
Day 7	21/03/16	33	25.1	7.9	49.1	70.9	-21.8
Day 9	23/03/16	22.6	22.6	0	80.3	80.9	-0.6
Day 15	29/03/16	27.1	19.6	7.5	52.2	71.4	-19.2
Day 21	04/04/16	32.6	22.1	10.5	43.5	73.7	-30.2
Day 28	11/04/16	24.1	23.1	1.0	66.4	77.1	-10.7

The pH of leaf wash solutions differed between locations, with the mean pH of leaf wash solutions from orchard samples significantly lower than those from the greenhouse (Figure 4.21). Correlation analyses did not reveal any direct relationship between pH and Cu^{2+} concentrations in leaf wash solutions for any sampling date (data not shown). The large initial differences in Cu^{2+} measured for leaves sprayed with either copper formulation seem most likely to be due to variables other than pH.

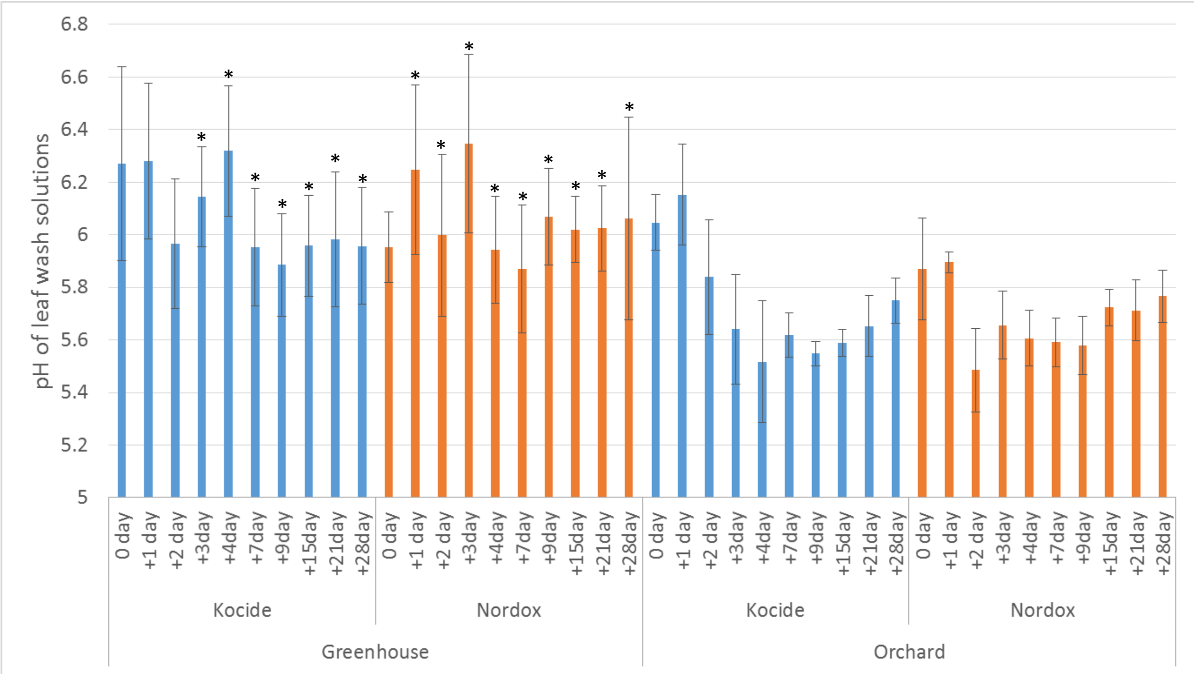

Figure 4.21: Comparison of pH of leaf wash solutions from leaves of potted plants positioned in a greenhouse and orchard over a 4 week period. Error bars denote ± SD. Significant differences between results from the greenhouse and orchard for each sampling time are indicated where * = p < 0.05 (n = 8).

Residual total copper deposits on leaves sprayed 28 days prior with Nordox® were significantly lower on orchard leaves (Figure 4.22). There was however no difference in total copper deposits remaining on leaves that had been sprayed with Kocide®.

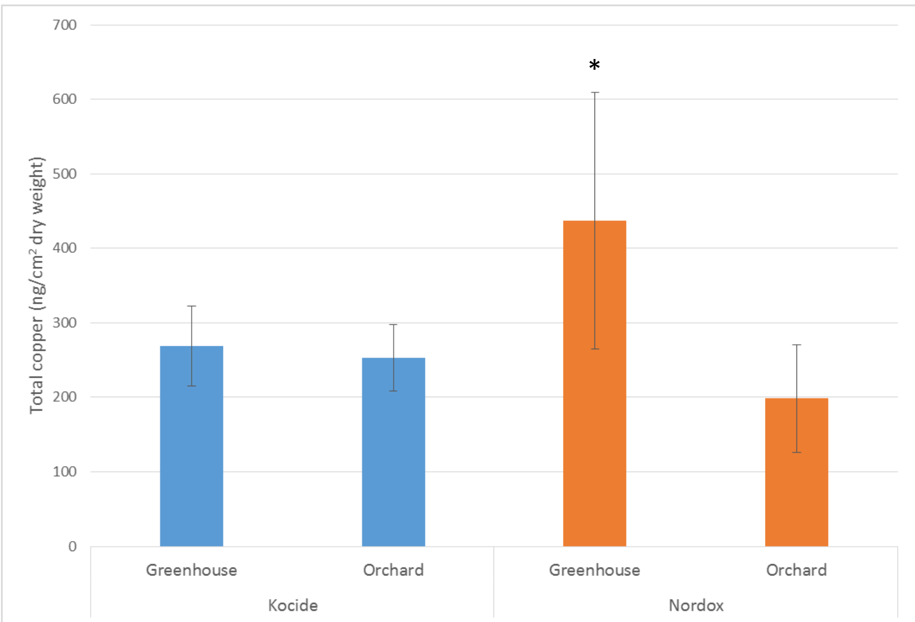

Figure 4.22: Total copper deposits remaining on leaves of potted plants 28 days after spray applications of Kocide® and Nordox®. Significant differences are indicated where *= p<0.05 (n = 8).

The condition of leaves on orchard plants at the end of the trail were relatively poor compared with those in the greenhouse, with a lot of brown patches and many starting to senesce (Figure 4.23)

Figure 4.23: Comparison of potted G3 plants in the greenhouse (top) and orchard (below) on Day 28 of the trial.

5. Discussion and Conclusions

It is evident from the data obtained during these trials that 'other' spray formulations will influence the chemical speciation of copper in solution and on leaf surfaces. This may be directly due to changes in the pH of the spray solution as is most likely the case with Citrox BioAlexin™ and Acadian. However, it is clear from our findings with potted G3 plants that pH may be only one factor that controls copper speciation as evidenced by the disparate interactions between Nordox® and Kocide® with Key Strepto™ where the availability of Cu^{2+} either increased or decreased in solution depending on the copper formulation.

The relatively small variation in leaf surface pH as a result of different spray applications may be due to the inherent buffering capacity and stability of the leaf surface, particularly over the short time frame of this trial where the plants used were tested in a greenhouse in relatively controlled climatic conditions. Therefore, the influence of pH on the speciation of copper on leaf surfaces may not necessarily correspond with that in solution. Chemical interactions on leaf surfaces involving binding with organic solutes and other complex chemical interactions will almost certainly influence the availability of Cu^{2+}.

Of the products tested with three different copper formulations Citrox BioAlexin™ consistently increased the concentration of Cu^{2+} in solution and/or on plant leaf surfaces. However, it should be noted that there may a greater risk of phytotoxicity with a decrease in pH and associated increase in the level of copper ions on plant surfaces, even though only limited signs of phytotoxicity were noted in these trials. There is perhaps potential for the use of Citrox BioAlexin™ for reactivating copper complexes on plant surfaces as an alternative to the reapplication copper, although the potential for phytotoxicity will need to be considered.

It is likely that Acadian will form organic complexes with copper ions, thereby reducing efficacy on leaf surfaces. Results of the Year 1 greenhouse trial suggested that a reduction in Cu^{2+} was only observed when Acadian was applied 24hrs prior to Nordox® and not as a tank mix or 24hrs after Nordox® applications. However, sampling took place 24 hrs after copper or Acadian were applied in combination. The results of the Year 2 sub-trial indicated that temporal variations in Cu^{2+} release from Nordox®-Acadian combinations on leaf surfaces may vary considerably as indicated by the differences in Cu^{2+} concentration on leaves sampled when dry, 24hrs and 48hrs post spraying. Results of the Year 2 trial revealed consistent reductions in Cu^{2+} release when Acadian was applied prior to, as a tank mix, or post Nordox® and Kocide® applications. Time of sampling did not influence the measured difference between Acadian-Kocide® treatments and controls and it is likely that Acadian will negatively influence the release of Cu^{2+} on leaf surfaces. Negative effects on Cu^{2+} release are likely if Acadian is applied up to and beyond 10 days either side of copper spray applications, depending on rainfall. The symptoms of phytotoxicity observed on leaves sprayed with Kocide® 10 days after an Acadian application may have been primarily due to leaf wetting and or the application of copper during the heat of the day. However, it is possible that phytotoxicity may have been somewhat exacerbated by the presence of Acadian as symptoms appeared to be significantly worse than observed for control leaves.

KeyStrepto™ also had the effect of greatly reducing copper availability in solution with Nordox® and Coptyzin. The availability of Cu^{2+} on leaves was also significantly reduced when KeyStrepto™ was applied 24hrs prior to Nordox®. The potential for bioefficacy may therefore be compromised if Nordox® or Coptyzin are applied after an application of this product.

The effects of Kasumin™ on Cu^{2+} release were very inconsistent, especially with Kasumin™-Kocide® interactions. The effects of Kasumin™ on Cu^{2+} release seem to be related to the timing of application. The overall indication for Nordox® is that Kasumin™ has the effect of reducing Cu^{2+} release when applied prior to copper and stimulating Cu^{2+} release when applied later. However, with a standard application as a tank mix with Kocide® onto an orchard canopy, this product seems to induce Cu^{2+} release on leaves over time, especially on the Hayward variety. The fact that these field trials were carried out on producing orchards, with inherent variability in spray deposition and uncertainty around prior spray deposition, the degree of difference between treatments and controls observed between orchards may not necessarily be solely due to kiwifruit variety.

Although Actigard™ when mixed with three different copper formulations in solution resulted in a reduction in Cu^{2+}, the availability of Cu^{2+} significantly increased when applied with Nordox® on leaves. However, as this product was not tested on leaves in combination with Kocide® and Coptyzin, the disparate results with Nordox® are difficult to explain. Further trial work may be required in order to validate these results.

There were conflicting results for Hi-Cane®, where the release of Cu^{2+} in solutions of Nordox® and Coptyzin was greatly reduced. Whereas, in solution with Kocide®, the concentration of Cu^{2+} increased. This product was not tested on potted plants however and it is unknown whether the effect will be consistent on plant surfaces. Hi-Cane® is applied to dormant vines when the risk of Psa-V infection is high due to weather conditions and damage to vines from pruning. As Hi-Cane® is not applied to leaves, the influence of this product on copper efficacy may need to be evaluated by an alternative method on mature vines in the field.

The results of sub-trials confirmed that differences observed between treatments and controls were due to product interactions and not leaf re-wetting. Time of sampling was not influential on observed treatment effects for Kocide® and the inconsistencies in treatment effects observed for Nordox® over three sampling periods may be due to the relatively insoluble nature of the product rather than time of sampling.

There is always inherent variability in environmental conditions and leaf surface chemistry which are influenced by temporal variations. This is especially so for orchards and explains why the product interaction studies were conducted in a more controlled environment. Differences in Cu^{2+} release were observed between both environments, however we were unable to ascribe variables to which these differences were attributed. Although there were variations in leaf pH, temperature and %RH between the two environments, these did not seem to correlate with Cu^{2+} release. Total copper deposits from Nordox® on leaves in the orchard were significantly lower than greenhouse leaves at the end of the trial. This could in part explain the significant differences in Cu^{2+} concentrations after this period of time. However, for Kocide® there was no significant difference in residual total copper deposits between each environment even though Cu^{2+} concentrations differed significantly. This also indicates that Kocide® may be more persistent than Nordox® on an orchard canopy after significant rain events. The observed trend of increasing Cu^{2+} release over time in the greenhouse could in part be due to phenological influences on leaf surface chemistry.

These trials have highlighted the need for growers to consider chemical interactions between copper formulations and other spray products when planning a spray programme. It is evident from the findings of this trial that certain spray products appear to greatly influence the availability Cu^{2+} on leaves and this will effect subsequent efficacy against bacterial disease, namely Psa.

6. References

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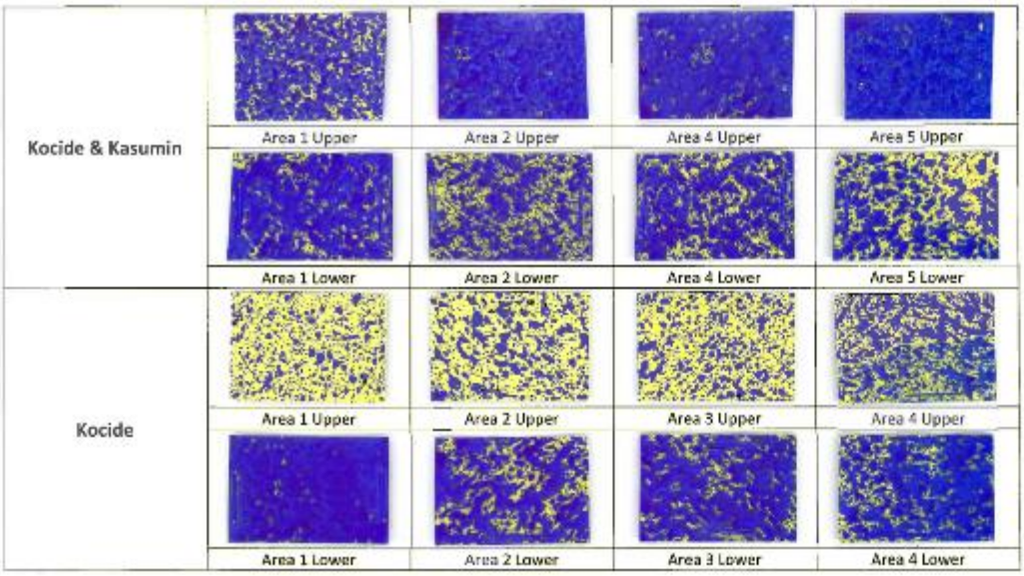
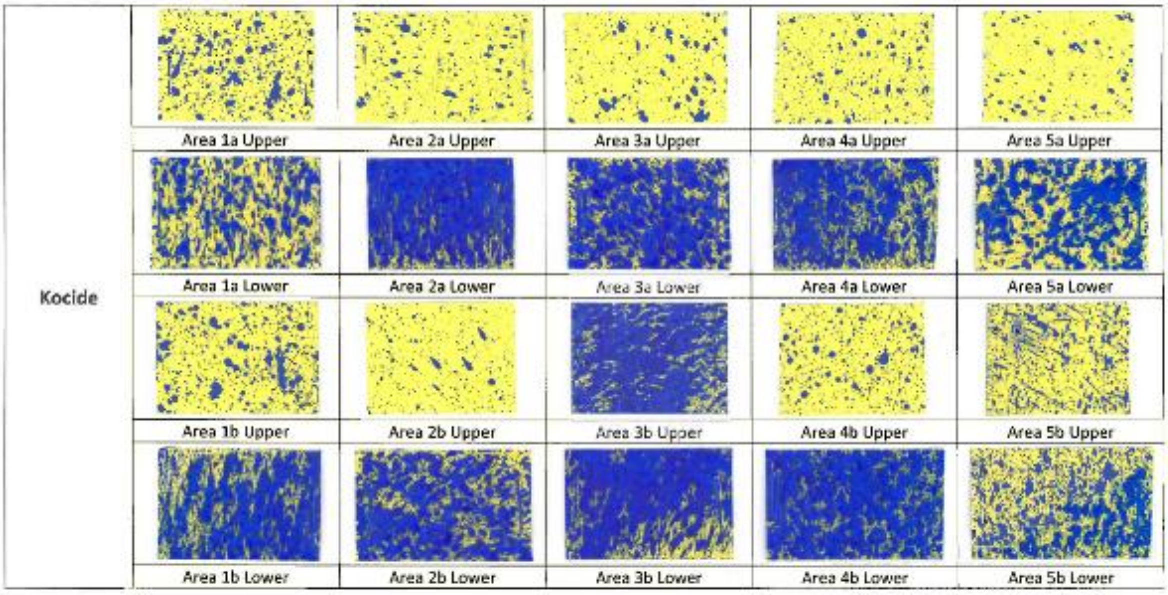
Appendix A: Water sensitive papers used in orchard trial

G3 Orchard



Hayward Orchard



Appendix B: Product interactions trials (Year 2) - temperature and humidity readings

Product	Treatment	Date	Temp (°C) range at time of spraying & sampling	%RH range at time of spraying & sampling
Greenhouse				
Acadian	-2d, -1d, Cu controls	19 Nov 2015	25 - 27	25 - 32
Acadian	-3d, -6d	23 Nov 2015	23 - 25	36 - 44
Kasumin	-2d	23 Nov 2015	23 - 25	36 - 44
Kasumin	tank mix, -2d, -1d, Cu	25 Nov 2015	29 - 32	34 - 42
Kasumin	-10d, -6d, -3d, Cu controls	30 Nov 2015	22 - 24	54 - 63
Citrox Bio.	+1d, Cu controls	2 Dec 2015	18 - 20	67 - 76
Citrox Bio.	+2d, +6d, Cu controls	3 Dec 2015	22 - 27	30 - 59
Citrox Bio.	+3d, Cu controls	4 Dec 2015	28 - 29	40 - 57
Citrox Bio.	+10d, Cu controls	7 Dec 2015	21 - 22	62 - 79
Acadian	+1d, Cu controls	9 Dec 2015	23 - 26	36 - 43
Acadian	+2d, +6d, Cu controls	10 Dec 2015	20 - 24	46 - 59
Acadian	+10d, Cu controls	14 Dec 2015	19 - 24	45 - 55
Citrox Bio.	+14d, Cu controls	15 Dec 2015	20 - 25	43 - 55
Kasumin	+1d, Cu controls	16 Dec 2015	20 - 26	42 - 62
Kasumin	+2d, +6d, Cu controls	17 Dec 2015	18 - 25	33 - 55
Kasumin	+3d, Cu controls	18 Dec 2015	20 - 29	29 - 50
Kasumin	+10d, Cu controls	21 Dec 2015	26 - 29	48 - 50
Citrox Bio.	+21d, Cu controls	11 Jan 2016	21 - 23	70 - 75
Citrox Bio.	+28d, Cu controls	18 Jan 2016	21 - 23	88 - 91
Citrox Bio.	-3d, -6d, Cu controls	25 Jan 2016	n/a	n/a
Citrox Bio.	-10d	15 Feb 2016	29 - 30	55 - 57
Acadian	-10d	15 Feb 2016	29 - 30	55 - 57
Acadian	tank mix	23 Feb 2016	21 - 23	73 - 77
G3 orchard				
Kasumin	tank mix	6 Oct 2015	14 - 18	63 - 75
Hayward orchard				
Kasumin	tank mix	20 Oct 2015	22 - 25	36 - 43

Appendix C: Phytotoxicity on leaves treated with Kocide®, Acadian® and Citrox BioAlexin™

<u>Acadian applied 10 days prior to Kocide®</u>

Acadian -10 days + Kocide (1)

Acadian -10 days + Kocide (2)

Acadian -10 days + Kocide (3)

Acadian -10 days + Kocide (4)

Acadian -10 days + Kocide (5)

Acadian -10 days + Kocide (6)

Acadian -10 days + Kocide (7)

Acadian -10 days + Kocide (8)

Citrox BioAlexin™ applied 10 days prior to Kocide®

Citrox Bio. -10 days + Kocide (1)

Citrox Bio. -10 days + Kocide (2)

Citrox Bio. -10 days + Kocide (4)

Citrox Bio. -10 days + Kocide (5)

Citrox Bio. -10 days + Kocide (6)

Citrox Bio. -10 days + Kocide (7)

Citrox Bio. -10 days + Kocide (8)

Acadian and Citrox BioAlexin™ treatment controls (Kocide® + DuWett®)

Control (1)

Control (2)

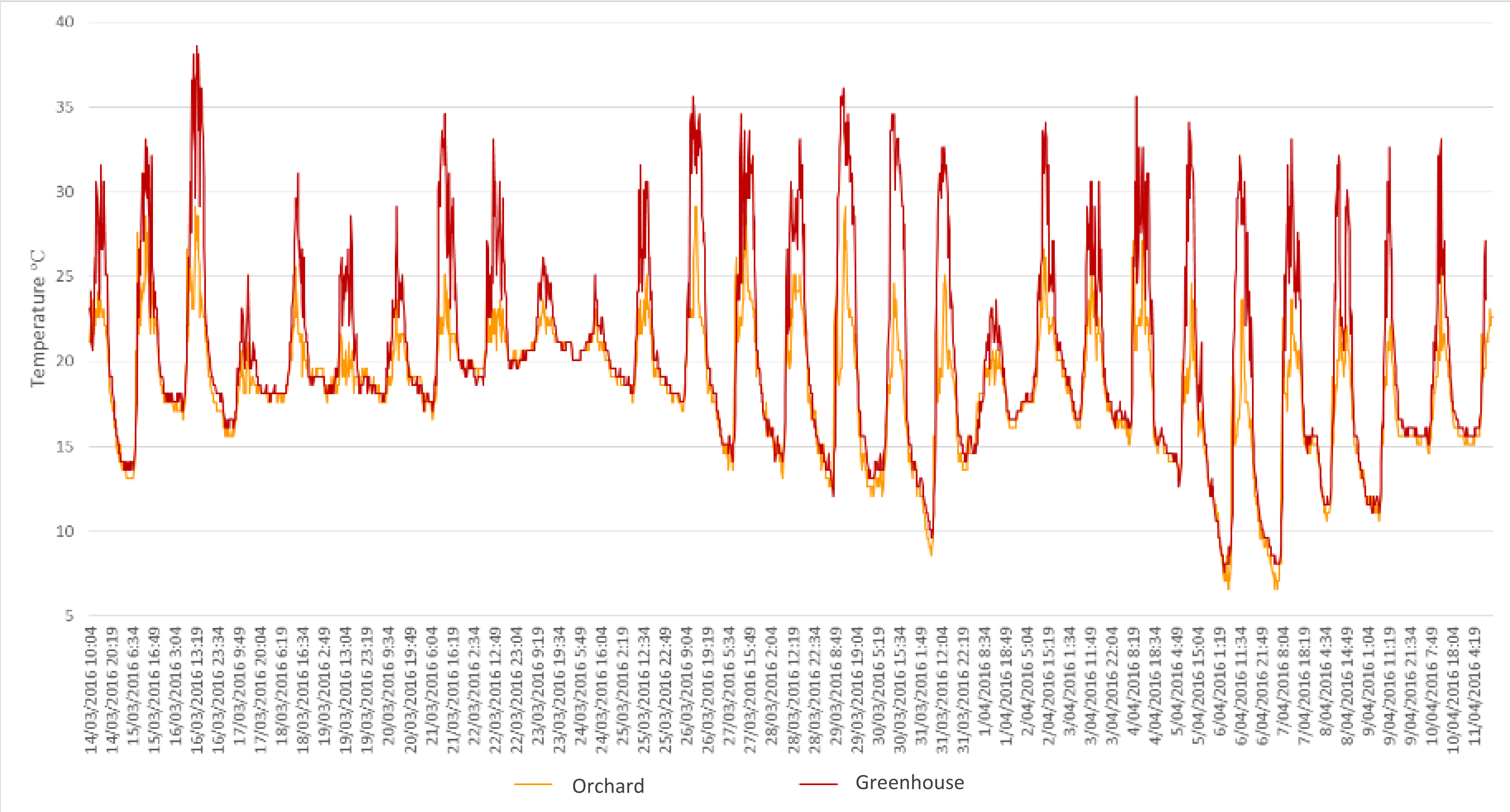
Control (3)

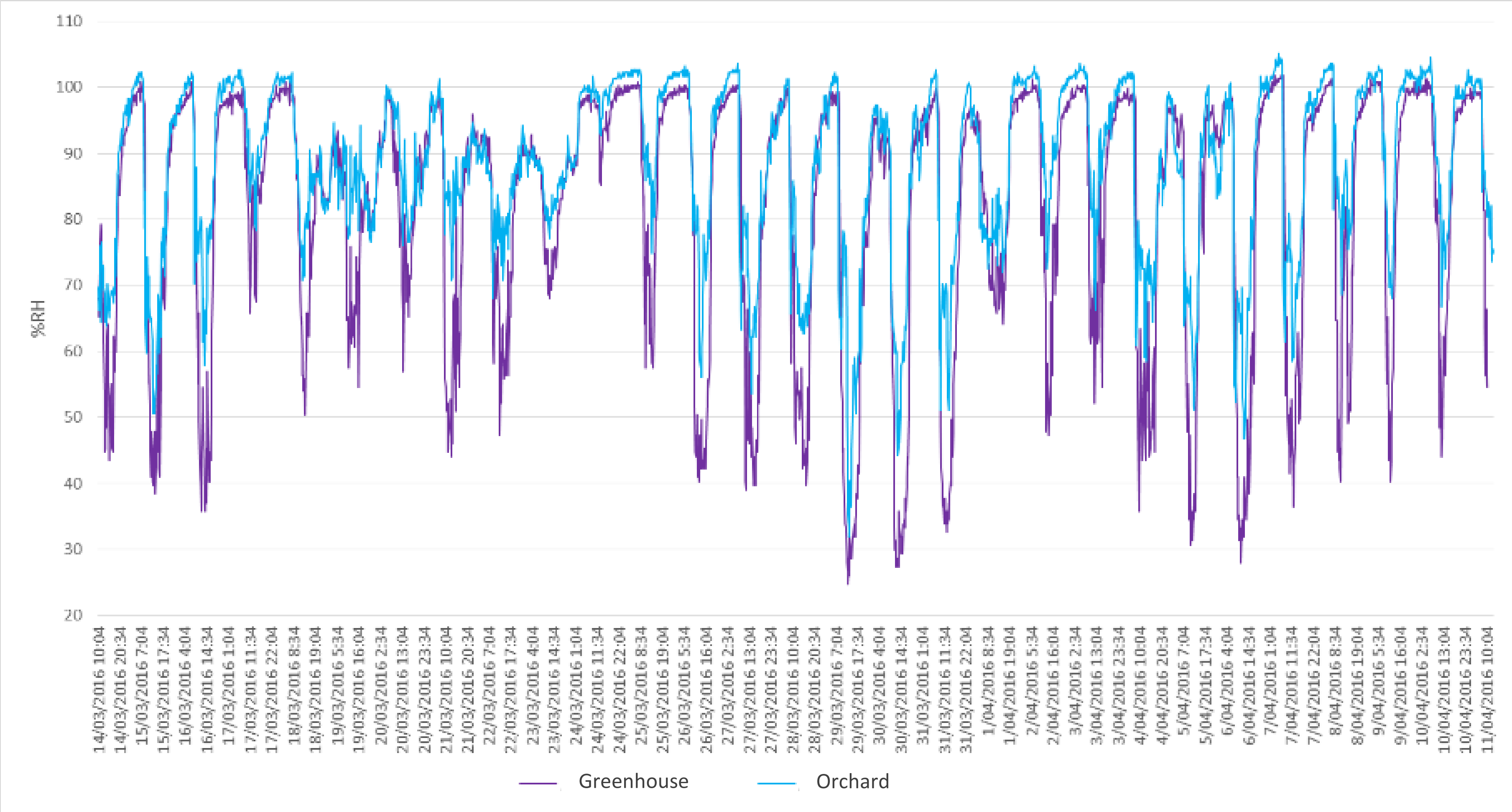
Control (4)

Control (5)

Control (7)

Appendix D: Effect of environmental conditions on Cu^{2+} release - temperature and humidity readings




%RH
110
100
90
80
70
60
50
40
30
20
Greenhouse
Orchard