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## **CP19008: Effect of tank mixing Kocide® Opti™ and Proclaim® on Psa control**

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May 2019

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## EXECUTIVE SUMMARY

### **CP19008: Effect of tank mixing Kocide® Opti™ and Proclaim® on Psa control**

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Plant & Food Research Ruakura

May 2019

Kiwifruit growers and spray contractors in New Zealand have many products to apply to their vines at key times of the year, such as spring. To save time, money and water, these products are often tank mixed. However, very little is understood about the impact of tank mixing on the effectiveness of the products, or the effect on the vines.

An artificially inoculated trial on potted *Actinidia chinensis* var. *deliciosa* 'Hayward' plants was carried out in the glasshouse with the following aims:

1. To determine whether tank-mixing of Kocide® Opti™ copper fungicide (used for *Pseudomonas syringae* pathovar *actinidiae* biovar 3 (Psa) control) and Proclaim® insecticide (used to control *Ctenopseustis obliquana* brownheaded leafroller (BHLR)) has a statistically significant effect on the incidence of Psa, versus individual use of either product
2. To measure residues of the active ingredients — copper for Kocide Opti and emamectin for Proclaim
3. To collect tissue for future analysis of host gene expression (by nanostring technology) in the event that significant treatment effects are observed.

Tank mixing fungicide Kocide Opti with Proclaim did not significantly affect Psa control, or have a noticeable influence on copper residues, but the emamectin residues increased markedly in the Kocide Opti + Proclaim mix (more than an additive effect), and this was the only treatment where emamectin was detectable 3 weeks post Psa inoculation. This suggests that a chemical interaction is occurring between the two pesticides to increase the residual concentration and longevity of the emamectin residue. Conversely, copper and emamectin residues decreased 2–4 fold with the addition of Duwett® to the Kocide Opti + Proclaim mix. This is most likely a physical rather than a chemical effect; considerable run-off was observed when DuWett was added to the spray mix, indicating that the applied water volume was excessive. This does not tend to occur in field conditions, where water volumes are modified and spray machinery enables accurate volumes to be applied. Further testing of residues resulting from tank mixed applications is recommended.

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# 1 INTRODUCTION

Kiwifruit growers and spray contractors in New Zealand have many pesticide products to apply to their vines at key times of the year, such as spring. To save time, money and water, these products are often tank mixed. However, very little is understood about the impact of tank mixing on the effectiveness of the products, or the effect on the vines. Two previously completed potted plant trials indicated that mixing Kocide® Opti™, a copper-based spray for control of *Pseudomonas syringae* pathovar *actinidiae* biovar 3 (Psa), and Proclaim®, an insecticide used to control *Ctenopseustis obliquana*, brownheaded leafroller (BHLR), significantly increased mortality of BHLR larvae (McKenna et al. 2017), but more Psa leaf spotting was also observed in *Actinidia chinensis* var. *deliciosa* 'Hayward' potted plants (HortEvaluation Limited 2018). This contrasting result requires further investigation and validation, as it has important implications for the practice of tank mixing these two products.

In addition, it is not known whether the observed effects were due to an interaction between the two pesticides (e.g. a binding of the two products), or to an effect on the kiwifruit immune response. If the phytohormone equilibrium was shifted towards elevation of the jasmonic acid (JA) pathway, this would favour BHLR control, since the JA pathway is implicated in plant defence to wounding and chewing damage caused by insects from the Lepidoptera order (Lazebnik et al. 2014). Conversely, upregulation of the JA pathway would likely cause suppression of the salicylic acid (SA) pathway, since expression of the two pathways is often inversely related (Pieterse et al. 2012) and this would favour Psa development (Cellini et al. 2014; Wurms et al. 2017b). To address both possible explanations, chemical residues will be measured and tissues sampled for subsequent gene expression using known markers of the JA and SA pathways (Wurms et al. 2017a; Wurms et al. 2017b).

The aims of this project were to:

1. Determine whether tank-mixing of Kocide Opti and Proclaim has a statistically significant effect on the incidence of Psa versus individual use of either product
2. Measure residues of the active ingredients — copper for Kocide Opti and emamectin for Proclaim
3. Collect tissue for future analysis of host gene expression (by nanostring technology) in the event that significant treatment effects are observed.

## 2 MATERIALS AND METHODS

### 2.1 Plant set up and application of treatments

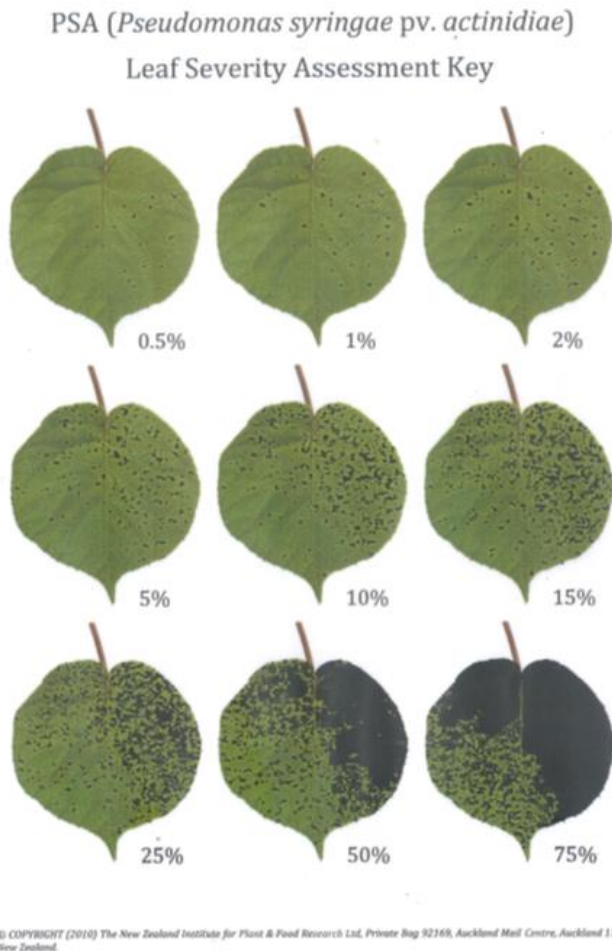
This study was completed on potted 'Hayward' plants (grown in 1.5 L pots) that were approximately 1 m tall at the start of the experiment. The plants were originally produced from tissue culture, and at approximately 2 months old they were hardened off in an outdoor shade area for 1 month, before being cut back to three leaf nodes and left for a further 2 months to allow fresh shoot growth before treatment applications. The plants were watered by drippers when in the shade house.

Each treatment was applied to 15 replicate plants by spraying both sides of every leaf to runoff, using 1 L spray bottles, with treatment application 1 day before Psa inoculation. There were five treatments: 1) water control; 2) Kocide Opti (0.7 g/L); 3) Proclaim (0.02 g/L); 4) Kocide Opti (0.7 g/L) plus Proclaim (0.02 g/L); and 5) Kocide Opti (0.7 g/L) plus Proclaim (0.02 g/L) plus DuWett® (0.05% v/v). Plants were allowed to dry before being returned to the shade house. The next day all plants were transferred to a containment glasshouse for inoculation with Psa.

Psa was applied as a  $2 \times 10^7$  colony forming units/mL spray to the underside of all the leaves. The plants were then placed into trays containing water (1–2 cm depth) and incubated under high humidity (enclosed tent flaps) for 2–3 days to allow infection to establish, followed by opening of small flaps on the plastic tents for the duration of the experiment. Pots within the tents were arranged in a complete randomised block design. The containment glasshouse was maintained at 15–25°C.

### 2.2 Psa disease assessments

Psa symptoms (leaf spot /necrosis) were assessed 14 and 21 days post inoculation. Up to six leaves were assessed per plant using a visual assessment key, developed in-house by The New Zealand Institute for Plant and Food Research Limited (PFR), which estimates percent necrosis (Figure 1). Results were averaged for each plant.



**Figure 1. *Pseudomonas syringae* pv. *actinidiae* (Psa) disease severity assessment diagrams for percentage infection on kiwifruit leaves, developed in-house by The New Zealand Institute for Plant and Food Research Limited.**

### 2.3 Analysis of chemical residues

Leaf samples were taken 1 day post-spray (just before Psa inoculation) and 3 weeks post-spray application for residue analysis of copper from the Kocide Opti spray and emamectin from the Proclaim spray. One fully expanded leaf was sampled per replicate plant at the same leaf position e.g. leaf 3 on 7 of the replicate plants and leaf 4 on the other 8 plants and then reverse of this for the sample at 3 weeks post spray application. Treatment samples were pooled into paper bags (i.e. 15 leaves/treatment) and were delivered immediately to Hill's Laboratories (Hamilton, New Zealand) for residue analysis. Unfortunately, when researching the procedures for this experiment, we were told by Hill's this amount of material would be sufficient for three replicate analyses (5 leaves each) per treatment, but upon delivery we discovered that there was insufficient material for treatment replication. For analysis of copper residues, the leaves were homogenised, dried for 16 h at 103°C, digested in nitric and hydrochloric acid, filtered, and then analysed by inductively coupled plasma mass spectrometry (ICP-MS), with correction for dry matter. Emamectin residues were detected as part of a multi residue analysis protocol (MR2) which includes solvent extraction, solid phase extraction column clean up and analysis by liquid chromatography with mass spectrometry using 2 mass analysers (LC-MS/MS).

## 2.4 Tissue collection for gene expression

Leaf samples for potential molecular analyses (not part of this project and subject to future stop/go analysis) were taken at 0 h, 24 h, 48 h, 72 h and 7 days after treatment application. At each time point, three leaves were removed from each of three plants per treatment. Care was taken to select leaves from the same position of each plant. Samples were taken from replicate plants #1–3 at 0 h, plants #4–6 at 24 h, plants #7–9 at 48 h, plants #10–12 at 72 h, and plants #13–15 at 7 days. At each sample time point, 2 x 20 mm discs were cut from each leaf, and the six discs from each plant were pooled for subsequent gene expression analysis. Samples were frozen at -80°C, ready for analysis, if required.

## 2.5 Statistical analysis

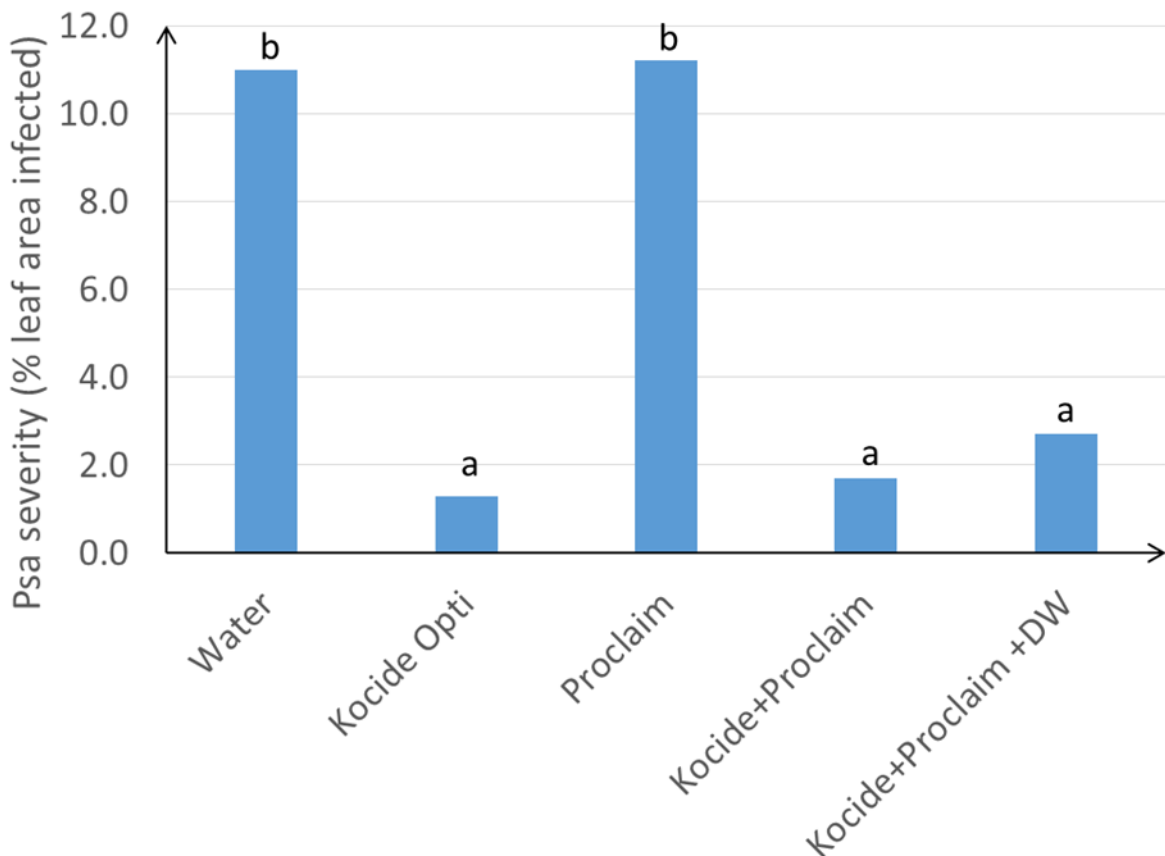
Analysis of the Psa assessment data was performed in SAS version 9.4 (SAS Institute, Cary, USA) using a generalized linear mixed model with Treatment as a fixed effect and the interaction between Treatment and Replicate as a random effect. Beta regression was used for the percentage area data.



## 3 RESULTS

### 3.1 Psa disease assessment

All treatments containing Kocide Opti significantly reduced Psa severity relative to the water control, and there was no significant difference between Kocide Opti and Kocide + Proclaim (Figure 2). Conversely, there was no difference in Psa severity between the water control and Proclaim (Figure 2). Addition of DuWett to Kocide Opti plus Proclaim slightly increased disease severity, but the effect was not statistically significant (Figure 2). Strong infection was observed, despite the experiment being carried out towards the end of summer, when plants are traditionally harder to infect, even in the glasshouse.



**Figure 2.** Percent leaf area of *Actinidia chinensis* var. *deliciosa* ‘Hayward’ kiwifruit leaves infected by *Pseudomonas syringae* pv. *actinidiae* (Psa) 21 days post spray inoculation with Psa ( $2 \times 10^7$  colony forming units/mL). Plants were sprayed with the following treatments 1 day prior to inoculation: 1) water control; 2) Kocide® Opti™ (0.7 g/L); 3) Proclaim® (0.02 g/L); 4) Kocide Opti (0.7 g/L) plus Proclaim (0.02 g/L); and 5) Kocide Opti (0.7 g/L) plus Proclaim (0.02 g/L) plus DuWett® (0.05% v/v). Treatments with different letters are statistically different, as indicated by Fisher’s Least Significant Difference (LSD), where  $p < 0.05$ .

### 3.2 Analysis of chemical residues

Although statistical comparisons were not possible due to the lack of replication (see Section 2.3), Table 1 nonetheless shows that copper residues were highest in the Kocide Opti treatment, decreased slightly in the Kocide Opti + Proclaim treatment, but were reduced by almost 2-fold in the Koicide Opti + Proclaim + Duwett combination (relative to the same treatment without DuWett), 1 day post treatment and by 2.5-fold after 3 weeks.

For the emamectin residues, 1 day post treatment, concentrations were highest in the Kocide Opti + Proclaim treatment and the amount found in this treatment was greater than could be obtained by adding together the residues from individual applications of Proclaim and Kocide Opti (Table 1). In contrast, Table 1 shows that the addition of Duwett to the Kocide Opti + Proclaim mixture reduced the emamectin residues by approximately 3.5-fold. At 3 weeks post spray application, Kocide Opti + Proclaim was the only treatment containing detectable emamectin residue.

**Table 1. Pesticide residues in *Actinidia chinensis* var. *deliciosa* ‘Hayward’ kiwifruit leaves, as measured by Hill’s Laboratories, Hamilton, New Zealand, associated with use of Kocide® Opti™ fungicide (copper residue), and Proclaim® insecticide (emamectin residue). Each treatment sample comprised 15 leaves (1 leaf per plant pooled from 15 replicate plants) and samples were collected 1 day and 3 weeks after treatment application. Pesticides were applied 1 day before inoculation with *Pseudomonas syringae* pv. *actinidiae* (Psa) ( $2 \times 10^7$  colony forming units/mL). Residues are given as mg residue/kg dry weight of leaf tissue.**

Sample date (days or weeks post treatment)	Residue type	Water	Kocide Opti (applied at 0.7 g/L)	Proclaim (applied at 0.02 g/L)	Koicide Opti + Proclaim	Koicide Opti + Proclaim + Duwett (0.05%)
1 day	copper	4.7	370	5.5	360	188
3 wk	copper	8.6	530	16.1	410	164
1 day	emamectin	0	0.018	0.11	0.28	0.08
3 wk	emamectin	0	0	0	0.27	0

## 4 DISCUSSION

Tank mixing Kocide Opti fungicide with Proclaim insecticide did not significantly affect Psa control, but emamectin residues increased markedly in the Kocide Opti + Proclaim mix (more than an additive effect), and this was the only treatment where emamectin was detectable 3 weeks post pesticide application. This indicates that a chemical interaction is occurring between the two pesticides to increase the residual concentration and longevity of the emamectin residue. Conversely, copper and emamectin residues decreased 2–4 fold with addition of DuWett to the Kocide Opti + Proclaim mix. This is most likely a physical rather than a chemical effect; as run-off was observed when DuWett was added to the spray mix, indicating that the applied water volume was excessive. DuWett label recommendations suggest reduced water rates, which are easily achieved in field conditions using an air-blast sprayer and a professional spray contractor, but are more difficult to achieve using a hand-held sprayer in a small glasshouse trial.

In this study, combining Kocide Opti with Proclaim did not adversely affect Psa control. A previous study recorded that Kocide Opti + Proclaim was less effective than other tank mixes at controlling Psa in 'Gold3' potted plants (HortEvaluation Limited 2018), but these data are inconclusive in that they were not compared to individual applications of each product and statistical analyses were not performed.

The greater emamectin residual concentration in Kocide Opti + Proclaim mix than in either product treatment on its own, along with the prolonged longevity is most likely due to a chemical interaction between the two products and may point to a synergistic reaction occurring. Chemical interactions between pesticides usually fall into one of three categories: additive (neutral interaction), synergistic (positive interaction) or antagonistic (negative interaction) (Penton Media Incorporated 2019). The chemical interaction is likely to improve efficacy of the Kocide Opti + Proclaim treatment against BHLR, but validation will require testing and statistical analysis of efficacy against BHLR, which is the proposed next stage of this research project. However, in support of the residue data obtained in this study, McKenna et al. (2017) tested the compatibility of pesticide mixes and found that mixing Proclaim with Kocide Opti significantly increased the longevity of toxicity against BHLR larvae. The implication of prolonged pesticide residue retention in fruit also needs to be considered in terms of fruit exceeding maximum residue limits, which may become a non-tariff trade barrier in certain export markets.

Mixing pesticides can offer the advantages of labour and time efficiency, reduced wear on equipment and improved efficacy of control. Conversely, it can also lead to a loss of efficacy and/or increased toxicity to humans, animals, the plants themselves and the environment. This is certainly an area requiring further investigation, particularly as we move into more integrated management systems.

## 5 RECOMMENDATIONS

- Test the effects of mixing Kocide Opti, Proclaim and Duwett on BHLR (this is proposed as a future part this project).
- Test other common pesticide combinations, including residue analysis, to determine if there are any chemical interactions and/or effects on the plant response, or on pest and disease control efficacy.

## 6 ACKNOWLEDGEMENTS

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