Can phosphorous acid be used to control *Neonectria ditissima* in New Zealand grown apples?

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Abstract European canker, *Neonectria ditissima*, is a worldwide apple tree disease killing shoots, branches and trees, and treatment with phosphorous acid is a possible control option. The effect of six postharvest phosphorous acid (PA) treatments on fruit residues the following season was studied in Tasman on two trial sites growing 'Scifresh' or 'Scilate' apple trees. Spray treatments consisted of number (0–3) and timing (early, mid and/or late) of PA applications. Additionally, leaf-scar wounds were artificially inoculated with *N. ditissima* spores at the 'Scilate' site on 1 and 8 June 2017 to determine disease control. Symptom expression was checked regularly between October 2017 and February 2018. None of the treatments caused a statistically significant reduction in the incidence of canker development compared with the control. Two or more PA applications resulted in PA residues in fruit, at harvest, the following season. Higher PA residues were found in fruit following early applications than with late applications. More applications of PA resulted in higher residues. This finding has important implications for exporting fruit to markets that have no tolerance for PA residues.

Keywords European canker, apple, inoculation, leaf scars, application, residue.

INTRODUCTION

European canker (EC) caused by the pathogen *Neonectria ditissima*, is a disease that has been reported from all regions of the world where apples are grown (CPC 2005, Beresford & Kim 2011). This pathogen produces cankers on shoots, branches and trunks of apple trees (Cooke 1999). These cankers can kill shoots and have a severe impact on plant growth, especially on young trees. Both the conidia and ascospores of *N. ditissima* infect the plant through wounds (Swinburne 1975; Xu et al. 1998) created either naturally or artificially. Amponsah et al. (2015) found that pruning cuts, followed by picking wounds and then leaf scars were the most important infection sites for apples grown in New Zealand.

Phosphorous acid (PA) is the main active ingredient in several commercial fungicides used for the control of root rots caused by *Phytophthora* and *Pythium* species. Phosgard™ (Grochem, Porirua, New Zealand) is one of these fungicides and contains 400 g/L of phosphorous acid as mono- and di-potassium salts in the form of soluble concentrates (Young 2016). A Brazilian study reported that a single application of PA (in the form of potassium phosphite) to 'Galaxy' apple trees caused a reduction in the expression of *N. ditissima* on sprayed and then excised and inoculated shoots (Boneti et al. 2015).

Two of the commonly used agrichemicals in New Zealand for *N. ditissima* control and prevention contain either captan (a trichloromethyl sulfenyl fungicide) or copper as the active ingredient. Both of these agrichemicals provide protectant activity only, so good coverage

of plant wounds is required to prevent infections by *N. ditissima* spores (Walter et al. 2015). In contrast, PA is a systemic product and wound coverage during spray application would be less critical if PA were shown to translocate to the area of the plant affected by the disease.

New Zealand apples grown for export are produced under an industry-wide programme (the Apple Future programme, a partnership programme between New Zealand Apples & Pears Inc. (formerly Pipfruit New Zealand Inc.) and New Zealand Ministry of Business, Innovation and Employment) that aims to generate fruit with ultra-low pesticide residues. The acceptable level of PA ranges from 0 to less than 1 ppm for most export markets so it is critical to understand the potential residues from applications of PA for disease control.

The aim of this study was to evaluate the efficacy of PA applications to mature apple trees in an orchard situation for EC suppression. Further, a review of PA residue levels in whole fruit from collections of fruitlets and fruit taken seven and ten months after application was undertaken to inform future use of PA on export apple crops.

MATERIALS AND METHODS

The first trial site was two rows of trees in a mature block of 'Scilate' situated on the Plant & Food Research (PFR) site at Whakarewa Street, Motueka. The second trial site was one row in a block of 12-year-old 'Scifresh' apple trees on the Waimea Plains, Richmond. For both sites, each plot consisted of two trees with one or more buffer trees between plots. Six treatments were established at both sites (Table 1), replicated five times. All applications used Phosgard™ (400g ai phosphorous acid/L), applied to whole tree with an Echo motorised knapsack sprayer, (model SHR-170SI, 2011) with two Teejet cone nozzles (model TYR8003VK). At both sites, applications occurred postharvest on one or more of the following dates 12, 22 and 30 May 2017 (Early, Mid and Late application treatments; Table 1), with a target water rate of 1500 L/ha. No leaf fall had occurred prior to the first and second applications at the PFR site and leaf fall was 2% at the time of the third application. In comparison, the tree phenology for the Waimea site for each application was 5%, 20% and 50% leaf fall, respectively.

On 1 June 2017, two days after the final phosphorous acid application, leaf-scar wounds were created on trees at the 'Scilate' PFR site only. These wounds were then inoculated with *N. ditissima* spores using the technique described by Walter et al. (2015). Three leaf scars were created per shoot, with four shoots per tree, to give a total of 12 inoculated wound sites per tree (24 wounds per plot/inoculation). This process was repeated on 8 June 2017 at the same site for the second inoculation. Shoots from the two inoculations were distinguished by colour coding. This gave a total of 24 wounds per tree, 48 per plot. Control plots were also inoculated with spores in the same manner as the spray plots. The spore suspension used for each inoculation was from the same batch (PFR batch number Wh4), with conidia

Table 1 Spray treatments containing the active ingredient (ai) phosphorous acid were applied on the 12 (Early), 22 (Mid) and 30 (Late) May 2017 to mature apple trees.

	Treatment	Total kg ai/ha	Total volume of product/ha
1	Control – Untreated	-	-
2	Early application	2	5
3	Early + Mid application	4	10 (5 L x 2)
4	Early + Mid + Late application	6	15 (5 L x 3)
5	Mid + Late application	4	10 (5 L x 2)
6	Late application	2	5

concentration of 3.9 x 10^4 spores/mL with 70–80% germination in the laboratory (originally sourced from canker lesions of 'Jonathon' and 'Scired' at the PFR Whakarewa site and stored frozen until use (Orchard et al. 2018). The 'Scifresh' Waimea site was not inoculated as it was a commercial orchard and did not want *N. ditissima* spores introduced.

An assessment of *N. ditissima* expression was conducted on 17 October 2017. This process involved monitoring each inoculated and all non-inoculated leaf-scar wounds (i.e. scanning the whole tree) and determining if any visible canker lesions had developed. The whole shoot was removed from any infection site that had developed a canker lesion even if just one inoculation site showed obvious disease symptoms (Walter et al. 2015). The few natural European cankers developing (generally on larger branches) were also recorded and removed. This process was repeated on 22 November 2017, 10 January and 14 February 2018. The numbers of lesions per treatment were calculated from these data. An analysis of variance (ANOVA) was performed on the total number of lesions removed (arising from artificial leaf-scar inoculation), using Analyse-it® for Microsoft Excel version 4.814, 2017 statistical computer package.

A sample of fruitlets was taken from both trial sites on 13 December 2017. This process involved selecting 10 fruitlets from each plot, pooling replicate treatments, to make a sample of 50 fruitlets, which was then bagged and placed in a commercial freezer at -18ºC. This process was repeated on 14 March (PFR site) and 3 April 2018 (Waimea site) with mature fruit by sampling five fruit from each plot. In May 2018, all fruitlet and fruit samples were sent to Hill Laboratories, Hamilton (NZS/ISO/IEC 17025:2005 accredited), where aqueous extraction, solid-phase extraction clean up and analysis by liquid chromatography-tandem mass spectrometry was conducted on whole fruit to detect and quantify any PA residues.

RESULTS

Overall, disease development from the inoculated leaf scars was low. The results of the ANOVA indicated no statistical difference between inoculation dates, therefore data were pooled. None of the PA treatments tested at the 'Scilate' site reduced lesion development compared to the unsprayed Control (Table 2). The Early +Mid treatment resulted in the lowest number European canker symptoms, with 0.5 lesions per shoot. A mean of 2.1 lesions per shoot occurred following the Late treatment, which was the

Table 2 The total number of European canker lesions with one more inoculation sites (three/shoot) developing symptoms following the inoculation of leaf scars on cultivar 'Scilate' sprayed with phosphorous acid on the 12 (Early), 22 (Mid) and/or 30 (Late) May 2017 at the Plant & Food Research site. SE refers to Standard Error of the mean. Means are based on five replicate plots consisting of 48 inoculated leaf scars on 16 shoots/2-tree plot.

Treatment	Number of Cankers	
	Mean	SE
Control	1.2	0.44
Early	0.9	0.38
Early + Mid	0.5	0.22
Early + Mid + Late	1.3	0.47
Mid + Late	1.3	0.54
Late	2.1	0.62
P-value	**0.2736**	

highest recorded. A mean of 1.2 canker lesions per shoot was recorded in the Control treatment (Table 2).

At the **Waimea** trial site, the PA residue levels for the Control and Late treatments were below the limit of detection (i.e. <0.4 mg/kg) in both the fruitlets and fruit (Table 3). The Early+Mid+Late treatment resulted in the highest fruitlet residue level at 1.3 mg/kg. In the mature fruit, 0.8 mg/kg was the highest residue level found, which was recorded for the Early+Mid and the Early+Mid+Late treatments. Analysis of fruit from the Mid+Late treatment found 0.6 mg/kg of PA. Residue values for all other fruit samples were below the limit of detection.

For the **PFR** trial site, the three-application treatment (Early+Mid+Late) resulted in the highest PA residue level in fruitlets with 2.9 mg/kg (Table 3). The Early and Mid+Late treatments led to 1.4 and 1.1 mg/kg residues found in fruitlets respectively. Phosphorous acid residues were below the limit of detection for the Control in both the fruitlet and fruit samples. The Late and Early treatments each resulted in the lowest levels of residues found in fruit, both with 0.5 mg/kg. Like that found in the fruitlets, the Early+Mid+Late treatment had the highest residues found in fruit (1.5 mg/kg). Higher residues in fruit may appear to correlate to lower EC lesion development but cannot be substantiated with the data obtained here. The R^2 value is 0.1996 but is not meaningful as insufficient residue data points (n=6) were obtained to undertake a valid correlation.

DISCUSSION

At the 'Scilate' PFR site, the overall percentage of inoculated leaf scars developing symptoms was 8.5%. This is similar to that found by Amponsah et al. (2015) where 7.2% and 10.6% of leaf scars developed cankers for 'Braeburn' and 'Scilate' respectively. The Early+Mid treatment resulted in the lowest expression of cankers but none of

Table 3 Phosphorus acid residues (mg/kg) from fruitlets and fruit harvested from either the Waimea or the PFR site after treatment with phosphorous acid applied Early, Mid or Late (12, 22 and 30 May 2017) postharvest.

		Residue (mg/kg)	
Waimea Site 'Scifresh'	Number of Applications	Fruitlets (13 Dec 2017)	Fruit (3 Apr 2018)
Control	0	<0.4	<0.4
Early	1	0.8	<0.4
Early + Mid	2	1.1	0.8
Early + Mid + Late	3	1.3	0.8
Mid + Late	2	1.2	0.6
Late	1	<0.4	<0.4
		Residue (mg/kg)	
PFR Site 'Scilate'	Number of Applications	Fruitlets (13 Dec 2017)	Fruit (14 Mar 2018)
Control	0	<0.4	<0.4
Early	1	1.4	0.5
Early + Mid	2	2.3	1.1
Early + Mid + Late	3	2.9	1.5
Mid + Late	2	1.1	0.9
Late	1	0.6	0.5

the treatments were statistically different. The data suggest that PA, in 2017, did not control *N. ditissima* infections. This is contrary to previous Brazilian work on 'Galaxy', which showed that PA reduced the incidence of *N. ditissima* disease development (Boneti et al. 2015) and earlier unpublished New Zealand research in 'Braeburn' where four PA applications (postharvest, early, mid, late) gave similar control to captan applications. However, fruit harvest occurred earlier (March) in the season for the 'Braeburn' and 'Galaxy' studies than for the 'Scilate' study reported here (April/May). Therefore, PA application commenced earlier in the autumn period when the apple trees were still actively growing. This combination of factors may play a role in PA uptake as could other differences in experimental design among the various studies. Nonetheless, in the 'Scilate' experiment, PA residues in fruit were found the following season, indicating that PA uptake had occurred. 'Scifresh' harvest also precedes 'Scilate' harvest and earlier PA applications are possible than those conducted here. We hypothesise that higher PA uptake and higher fruit residues will be likely in 'Scifresh' trees (further discussed below) as a result of earlier PA applications and greater plant growth at the time of application.

No apples were present at time of the PA applications late in the 2017 season yet detectable PA residues were found in fruit of sprayed trees but not in unsprayed trees the following year (>300 days after application, Table 1). This finding clearly reflects the systemic and persistent nature of phosphorous acid. Malusa and Tosi (2005) also found long-term persistence of PA in plant storage organs, including apple fruit, up to two years after application. In that same study, applications were used post-flowering and residues exceeded the acceptable limit (<1 ppm) under EU legislation. The PA residues found from the two sites in this trial were lower than those permissible for apple exports to Europe. However, some export markets have no minimum residue limit set for PA, so non-detectable residues become the default requirement. The results from this trial indicate that any more than one application of PA will result in apples with detectable PA residues the following year, and consequently this crop would be at risk of exclusion from some export markets. It is not possible to guarantee that even one application of PA will result in fruit from the following season that are residue free. These findings have important implications for New Zealand apple exports.

In both trial sites, more applications of PA resulted in higher residues in both fruitlets and fruit. However, applications that were applied later resulted lower residue levels. This outcome is probably associated with plant physiology. Applications of PA applied to foliage would be less likely to be absorbed if the trees had already entered dormancy and leaf fall was occurring. This could explain why the Late treatment at the Waimea site resulted in no detectable PA residues in fruitlets or fruit as that application occurred at 50% leaf fall. Less absorption was likely with half the leaves missing compared with when only 5% of the leaves had fallen. At the PFR site, the differences between plant phenology were less pronounced between the Early and Late treatments i.e. postharvest and 2% leaf fall. However, even this minor difference in plant phenology at the PFR site resulted in twice the amount of PA residue in fruitlets from the Early compared with the Late treatment.

Overall, higher PA residues were found in fruitlets and fruit for all treatments at the PFR site compared with the Waimea site. All applications were applied at an earlier plant phenology stage at the PFR site compared with the Waimea site. As discussed previously, it is reasonable that more PA uptake occurred and hence higher residues were found because trees at PFR were still growing when PA treatment occurred. This outcome indicates that any PA needs to be applied before, or close to, the start of leaf fall to maximise uptake and disease suppression. Applications at 50% leaf fall appears to have minimal fungicide effect and probably very little uptake. Also, disease control potential becomes irrelevant if PA residues remain in fruit for one or, potentially, two seasons.

CONCLUSIONS

In conclusion, in this study, *N. ditissima* disease control of leaf-scar infections using PA could not be ascertained. More applications of PA resulted in higher residues in fruit, despite applications occurring postharvest and well before any fruit were formed. Plant phenology clearly has an impact on PA absorption, with any treatment applied close to or at leaf fall having less uptake and, therefore, less residue than if applied earlier. Even one application of PA can produce detectable residues in the following year's apple fruit at harvest. These results have important implications for the New Zealand apple industry when exporting fruit to markets that have little or no tolerance for PA residues. Therefore, the use of PA, postharvest for control of *N. ditissima*, is not recommended in New Zealand apple production.

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