Residue testing of avocado trees treated with phosphorous acid-based trunk injections for the control of *Phytophthora cinnamomi*

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Phytophthora cinnamomi Rands is an important fungal root disease that causes decay and yield loss in avocado (*Persea americana* Mill). Phosphorus acid has proved effective at controlling *Phytophthora cinnamomi* in avocado orchards. In South Africa, the maximum residue limit (MRL) for phosphorous acid in avocado fruit is set at 50 mg/kg; however, growers have difficulty maintaining this MRL when applying phosphorous acid as trunk injections. This study was conducted to help establish the lowest possible residue levels that may be obtained with regular use of phosphorous acid-based trunk injections against *Phytophthora cinnamomi*. This study also determined whether phosphorous acid build-up occurs in 'Pinkerton' and 'Hass' avocado trees treated with more than one phosphorous acid-based trunk injection. The trial was conducted in the Politsi region, in three 'Hass' orchards of ages 4, 9 and 23 years and three 'Pinkerton' orchards of ages 4, 8 and 18 years. The trial was laid out as a randomized complete block design, replicated five times, in five tree plots. The trial included five phosphorous acid-based trunk treatments applied as injections; untreated trees were used as controls. Trunk injections were applied in November, February and May. Residue samples were taken 30 and 60 days after the first injections (DAFI) and again 30 days after the second injections (DASI). Samples were sent for phosphorous acid residue analysis. Results indicate that build-up of phosphorous acid may occur in 'Hass' and 'Pinkerton' trees depending on tree age and number of injections applied over the season. The report mostly discusses the data collected from the second season due to the high variance and some discrepancies in the data from the first season. Although some trends were observed over the seasons, confirmation of these trends by repeating the trial is required before more accurate recommendations can be made.

INTRODUCTION

Phytophthora cinnamomi Rands is a soil-borne pathogen that causes Phytophthora root rot in avocado trees. Symptoms of Phytophthora root rot infection include degradation and rot of the roots that can lead to stunting, foliage decline at the top of the tree and branch die-back. Tree health decline may also affect fruit size and yield (Pak and Everett, 2001).

P. cinnamomi is most commonly treated with concentrated solutions of phosphorous acid applied as trunk injections using syringes (Horner and Jensen, 2004). Following trunk injections, phosphorous tends to move up the tree into the leaves before moving downwards to the roots. The concentration in the roots then becomes relatively high when compared to other parts of the tree and it is in this way that the spread of *P. cinnamomi* may be inhibited (Pegg *et al*., 2002). Phosphorous acid has good efficacy against Phytophthora root rot and a single trunk injection may be adequate to control Phytophthora root rot for 6 months (Faber and Downer, 2007; Giblin *et al*., 2007).

The European Union has set the acceptable MRL (maximum residue limit) for phosphorous acid in avocado fruit at 50 mg/kg for exports to EU markets. Although the trunk injection technique has proven the most effective method for controlling *P. cinnamomi* in avocado, the lowest possible MRL that can be expected from regular applications of phosphorous acid using this method has not yet been established.

The aim of this study was to determine whether phosphorous acid accumulates over time in avocado trees treated with more than one phosphorous acid trunk injection. The study also aimed to determine the lowest possible MRL that can be obtained in 'Hass' and 'Pinkerton' avocado fruit after regular trunk injections with phosphorous acid.

MATERIALS AND METHODS

The trial was conducted in three 'Hass' orchards of ages 4, 9 and 23 and three 'Pinkerton' orchards of ages 4, 8 and 18 in the Politsi region (23°45'54.0''S; 30°07'53.7''E; Köppen-Geiger bioclimatic zone: Cwa)

Table 1. Trial layout – 'Hass'.

4.1	5.2	1.3	6.4	3.5
6.1	1.2	4.3	3.4	2.5
3.1	4.2	6.3	2.4	5.5
1.1	2.2	5.3	4.4	6.5
5.1	3.2	2.3	1.4	4.5
2.1	6.2	3.3	5.4	1.5

Table 2. Trial layout – 'Pinkerton'.

1.3	3.5	4.1	6.4	5.2
4.3	2.5	6.1	3.4	1.2
6.3	5.5	3.1	2.4	4.2
5.3	6.5	1.1	4.4	2.2
2.3	4.5	5.1	1.4	3.2
3.3	1.5	2.1	5.4	6.2

Table 3. Treatments and application rates.

Treatment description	Product/Active ingredient	Dosage	Treatment dates	Product volume per injection (mL)
1. Untreated control	-	-	-	-
2. M5 – 1X	Phosphorous acid	14 g/L	Nov & Feb	20
3. M5 – 1X	Phosphorous acid	14 g/L	Nov, Feb & May	20
4. M5 – 2X	Phosphorous acid	28 g/L	Nov & Feb	20
5. M5 – 2X	Phosphorous acid	28 g/L	Nov, Feb & May	20
6. Avoguard 500 SL	Phosphorous acid	500 g/L	Nov & Feb	5

Table 4. Application dates and weather information on the day of spraying.

Application no.	Date	Weather information
1	1/12/2015	Clear; Temperature: 35.1°C, Relative humidity: 26%
2	16/02/2016	Clear; Temperature: 34.8°C, Relative humidity: 31%
3	18/05/2016	Overcast; Temperature: 22.3°C, Relative humidity: 60%
4	8/11/2016	Partly cloudy; Temperature: 32.1°C, Relative humidity: 31%
5	9/02/2017	Partly cloudy; Temperature: 30.1°C, Relative humidity: 50%

known to be affected by *Phytophthora cinnamomi*. Each orchard consisted of three-tree plots, replicated five times in a randomised complete block design and laid out as indicated in Tables 1 and 2.

First treatment applications commenced in November 2015 in the form of trunk injections (Table 3). Each tree received four injections equidistant from one another around the trunk with each application. Application dates and weather information is depicted in Table 4.

Residue samples were taken from an indexed branch on each tree, 30 and 60 days after the first injection (DAFI) and again 30 days after the second injection (DASI) in the first season. No samples were collected at harvest in the first season as fruit was harvested before samples could be collected. In the second season, residue samples were collected at 30 and 60 DAFI and at 30 DASI and lastly, at harvest. Fruit samples were sent to an accredited analytical laboratory in South Africa and tested for phosphorous acid. A different laboratory was used for each of the seasons, due to discrepancies in the results from the first laboratory.

Due to unforeseen circumstances and discrepancies in the data of season 1, the data is not included in this report. Data collected from season 2 are depicted in the report and discussed.

RESULTS AND DISCUSSION

Pinkerton

The MRL data (Figure 1) obtained from the second lab used in season two, shows varying accumulation and breakdown of phosphorous acid in 'Pinkerton' trees of various ages. Different aspects such as tree age, dosage rate and number of applications appear to interact differently resulting in varying results.

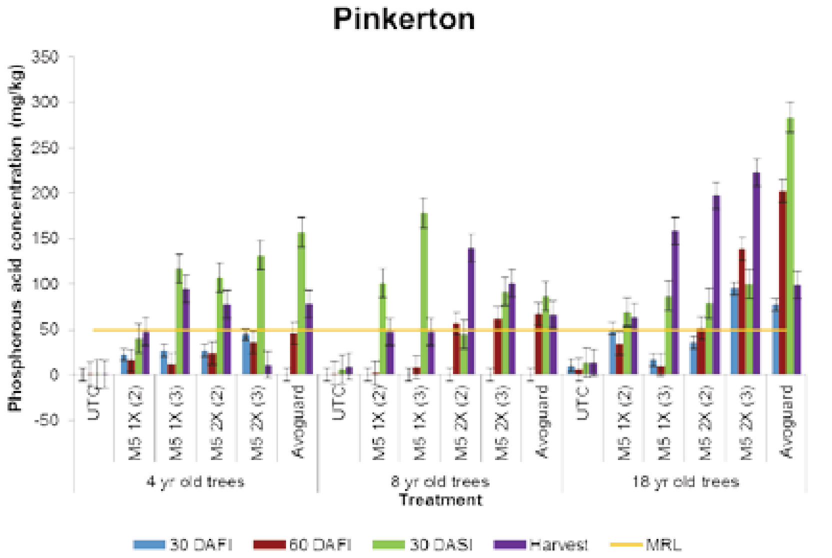
When M5 was applied to young trees (4 years) at a single dose rate twice per season that phosphorous acid accumulated steadily from the first application. The residues detected at harvest were, however, still below the accepted phosphorous acid level. The single dose rate application applied three times per season gave very similar results to the double dose rate applications (both 2 and 3 applications per season) and Avoguard. With these dose rates, phosphorous acid did not break down sufficiently by the time of harvest to reach levels below the accepted MRL, except for the M5 double dose rate applied three times per season. It is not understood why the double dose rate applied three times per season broke down to below the accepted MRL, whilst the same dose rate applied only twice per season did not break down in a similar way. Unfortunately these results cannot be compared to the previous season's MRLs.

Phosphorous acid appears to break down slightly better in the 8-year old trees when looking at the single dose rate applications. Phosphorous acid levels increased to peak levels after the second applications and decreased well enough to fall below the accepted MRL at harvest. The single dose rate applications of M5 applied two and three times per season, broke down better than the Avoguard applications applied twice per season. The double dose rate applications of M5 did not break down sufficiently and remained very high until harvest.

In the 18-year old trees neither M5 nor Avoguard broke down to acceptable levels during the season. This might indicate that continuous use of phosphorous acid during the lifespan of the trees does lead to an accumulation of residues in the trees that moves into the fruit in each growth cycle. This thought is supported by the low levels of phosphorous acid detected in the untreated trees. These residues are most likely from the phosphorous acid applications applied to the trees in the seasons before the trial was started. As seen here, it may take several years for phosphorous acid to completely break down in the trees.

Hass

Figure 2 depicts the MRL data for fruit collected in the second season from the 'Hass' orchards. The 4-year old 'Hass' orchard shows that both the single and double dose rate treatments applied only twice in the season broke down well enough to fall below the accepted MRL by the time of harvest.



* The 2 and 3 in brackets behind the treatment name denote the number of injections applied as laid out in Table 3.

** Yellow line denotes 50 mg/kg the acceptable MRL (maximum residue limit) for phosphorous acid in avocado fruit exports to EU.

Figure 1. Concentration of phosphorous acid in 'Pinkerton' fruit per treatment in season 2.

The single and double dose rate treatments applied three times per season showed a steady increase in phosphorous acid residues in the fruit and no sign of decline. The levels were above the accepted MRL, but the levels were still much lower than that of Avoguard. Avoguard seemed too increased rapidly after the second application in the season and kept on increasing up until the harvest samples were collected.

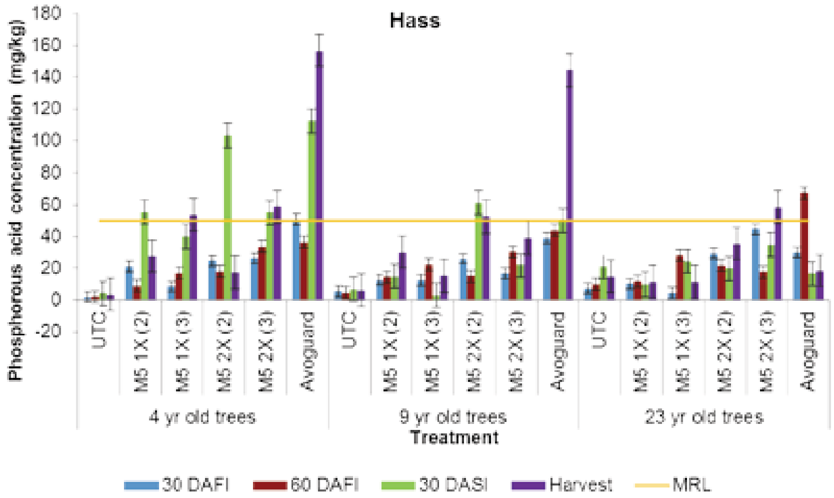
The 9-year old trees showed a similar trend. Most of the M5 treatments remained below the accepted MRL. This could indicate that 'Hass' trees of this age more readily breaks down the applied phosphorous acid, instead of translocating the acid to the fruit. Avoguard again appears to keep on accumulating throughout the season, resulting in harvested samples being well above the accepted MRL.

A different trend is observed in the 23-year old trees. Single dose rate treatments applied twice and three times per season showed very little accumulation of phosphorous acid in the fruit samples. It appears that the phosphorous acid breaks down very well by the time of harvest. The double dose rate applied twice per season showed a decline in phosphorous acid levels after the first and second application, but an increase in levels by the time of harvest. These levels were still well below the accepted MRL. The double dose rate treatment applied three times per season showed a similar trend, although the levels at harvest were above the accepted MRL. Avoguard broke down very well during the season and at harvest the levels of phosphorous acid in the fruit was below the accepted MRL.

CONCLUSION

Pinkerton

Only the single dose rate applied twice per season broke down to below the acceptable MRL levels in the 4-year old trees. The 8-year old trees seemed to break down the single dose rate treatments better than the younger trees. The double dose rate treatments did not break down to acceptable levels. The older trees (18 years) indicated that the phosphorous acid did not break down to acceptable levels at all. This could potentially indicate that trees in the prime of their production, break down the phosphorous acid more readily, while the older trees do not. This could be due to years of



* The 2 and 3 in brackets behind the treatment name denote the number of injections applied as laid out in Table 3.
** Yellow line denotes 50 mg/kg the acceptable MRL (maximum residue limit) for phosphorous acid in avocado fruit exports to EU.

Figure 2. Concentration of phosphorous acid in 'Hass' fruit per treatment in season 2.

phosphorous acid accumulation. This might mean that older trees might not need to be treated with phosphorous acid injections every season, or that the number of injections could be reduced to a single injection at a critical root-rot infection time. In order to confirm these trends, the trial needs to be repeated before these recommendations can be applied.

Hass

The 'Hass' orchards showed a similar trend as the 'Pinkerton' orchards with regards to the youngest trees tested. The older the trees the better the breakdown of phosphorous acid (M5) seemed to become. Avoguard did not break down well in the two younger orchards, but did break down very well in the oldest orchard.

From these results it would appear that 'Pinkerton' and 'Hass' trees reacted differently to the treatments as the ages of the trees increased. Overall, it appears that 'Pinkerton' accumulates phosphorous acid at higher levels compared to 'Hass', especially as the trees get older. Older 'Hass' trees, however, indicates higher phosphorous acid break down between application. The dosage rates and number of applications per season should, however, be carefully considered for each cultivar in order to avoid exceeding allowable phosphorous acid MRL levels at the time of harvest.

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