Optimizing foliar phosphonate sprays for the preventative management of Phytophthora root rot on avocado

– Preliminary report

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ABSTRACT

Foliar ammonium- and potassium phosphonate sprays were evaluated as a replacement for phosphonate trunk injections that are currently registered and used for the preventative management of Phytophthora root rot on avocado in South Africa. Two orchard trials were conducted in the 2017/18 season where foliar sprays were only applied in fall after harvest, as a strategy to reduce fruit residues. The efficacy of the foliar sprays (four or five sprays) was evaluated by measuring root phosphite (breakdown product of phosphonates in plants) concentrations and *Phytophthora cinnamomi* zoospore production from roots. Potassium- and ammonium phosphonate sprays applied as four or five 0.5% a.i. sprays at weekly intervals yielded root phosphite concentrations, 4 and 20 weeks after application, which were not significantly lower than the registered curative (0.5 g a.i./m^2) trunk injection treatment. In the one trial, the ammonium phosphonate foliar sprays consistently yielded significantly higher root phosphite concentrations than the potassium phosphonate foliar sprays. However, in the other trial this was only true for the 20 weeks after application time point. All the foliar phosphonate spray treatments were able to significantly reduce the amount of *P. cinnamomi* zoospores released from roots relative to the untreated control, and at a level that did not differ significantly from the curative- (0.5 g a.i./m^2) and preventative (0.3 g a.i./m^2) trunk injection treatments. The ammonium- and potassium phosphonate foliar sprays only caused mild foliar phytotoxicity at a low incidence when applied at the 0.5% a.i. dosage. The ammonium phosphonate foliar sprays tended to be slightly more phytotoxic than the potassium phosphonate foliar sprays. The trials will be continued until April 2018 to further quantify phosphite in roots up until 11 months after the first phosphonate applications were made, and to determine fruit residues at harvest.

INTRODUCTION

Phytophthora cinnamomi Rands is the causal agent of Phytophthora root rot in avocado (Pegg *et al.,* 1987). The pathogen is an oomycetous soil-borne pathogen of woody and ornamental plants (Linde *et al.,* 1999), causing severe crop losses in several avocado producing regions of the world, including South Africa (McDonald *et al.,* 2007; Engelbrecht *et al.,* 2013). Phytophthora root rot is especially problematic in regions where favourable environmental conditions prevail, such as prolonged wet periods in soil, where *P. cinnamomi* can reproduce through the production of a vast number of asexual bi-flagellate zoospores that are released from sporangia (Hardham, 2005).

The motile zoospores of *Phytophthora* species found in soil and water sources can be quantified using baiting techniques (Greenhalgh, 1978). The baiting technique involves detection and quantification of zoospore infections of leaf- or fruit baits. The method involves the exposure of leaf baits to water or soil slurries potentially containing the pathogen, where after the baits are plated onto a *Phytophthora* selective medium such as PARPH-V8 medium, followed by morphological characterization of hyphal growth emitting from the leaf baits. The percentage infected leaf baits is a semi-quantitative indication of the number of infective propagules (zoospores) of the pathogen that were present in a sample. Although leaf baiting has mainly been used for the semi-quantitative analyses of *Phytophthora* spp. in water and soil samples, it can potentially also be used to assess the amount of root colonization by

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the pathogen. The latter is important when evaluating management strategies.

The effective management of Phytophthora root rot can be achieved using phosphonate fungicides (Darvas *et al.,* 1984; Aryantha and Guest, 2004). Phosphonate fungicides include a range of salts (Na^+, Mg^{2+}, Cu^{2+}, Zn^{2+} and Al^{3+}) and esters of phosphorous acid. In addition to the aforementioned salts, a NH_4^+ salt of phosphorous acid is also registered in South Africa for use on several crops. For adequate control of *Phytophthora* spp. using phosphonates, it is important to apply phosphonates in the correct application window, when roots are a sink for photoassimilates since phosphonates are translocated in a source sink manner (Whiley *et al.*, 1995). Sufficient translocation of phosphite to roots is important, since phosphite is the active compound involved in Phytophthora suppression (Whiley *et al*., 1995; Hardy *et al*., 2001). Although some articles have been published showing a correlation between phosphite concentrations in plant tissue and pathogen suppression (Coffey and Joseph, 1985; Aryantha and Guest, 2004), there is as of yet not a clear indication of the specific amount of phosphite required for suppression of *Phytophthora* spp. For *P. cinnamomi* on avocado, only information from Australia is available, where a critical root phosphite concentration of more than 25 $\mu g/g_{FW}$ has been established using long term commercial root phosphite concentration data (Thomas, 2008). However, experimental evidence on a critical root phosphite level required for *P. cinnamomi* in avocado roots is still lacking.

In the South African avocado industry, phosphonate trunk injections are used in a preventative strategy against root rot. Phosphonate trunk injections are applied twice per growing season as was first reported by Darvas *et al.* (1983), to obtain effective Phytophthora root rot control. The registered trunk injection dosages in South Africa for curative control of root rot is 0.5 g a.i./m^2, whereas 0.3 g a.i./m^2 is used in a preventative strategy.

Foliar phosphonate sprays have recently been reported as having the potential for replacing phosphonate trunk injections, which have become very costly and can be damaging to avocado trees (McLeod *et al.,* 2018). In Australia, foliar potassium phosphonate sprays are used by most growers for managing root rot preventatively (Thomas, 2008). It is not advisable to use foliar sprays curatively for the treatment of trees that show symptoms, since diseased trees do not contain enough foliage for uptake of foliar phosphonate sprays. In South Africa, McLeod *et al.* (2018) evaluated ammonium- and potassium foliar phosphonate sprays applied as a split dosage in fall (three 0.6% sprays) and summer (two 0.5% sprays) in the 2015/16 season. The foliar sprays yielded root phosphite concentrations that were comparable to two trunk injections applied at 0.5 g a.i./m^2. However, fruit residues of the trunk injection treatment and the foliar sprays in some trials exceeded the maximum residue level of 50 mg/kg (McLeod *et al.,* 2018). This was most likely caused by applications that were made in summer (November/December) when small fruits were present on trees, since small fruits are a strong sink for phosphonates. Therefore, subsequent trials in the 2016/17 season were conducted that evaluated only one 0.5% foliar spray applied in summer and three 0.5% sprays applied in fall. This strategy yielded root phosphite concentrations that were comparable to the trunk injection treatment of 0.5 g a.i./m^2, from May (7 weeks after application) to December (32 weeks after application) in the two orchard trials that were conducted (McLeod *et al.,* 2017). However, at later time points (47 weeks after application) all the foliar spray treatments in the one trial had significantly lower root phosphite concentrations than the trunk injection treatment (unpublished data). This could be due to summer foliar sprays being translocated less effectively to roots since, although a root flush occurred at the time of application, small fruits on the tree might reduce the amount of phosphite translocated to roots. Alternatively, a total of only four annual foliar sprays might not be sufficient, and five sprays will be required. The strategy of reducing the summer foliar sprays to only one spray in the 2016/17 trials was effective in yielding fruit residues that were below the MRL for all foliar spray treatments. In comparison, in some of the same trials the registered curative trunk injections resulted in fruit residues that exceeded the MRL (unpublished data).

The aim of this study was to further optimise foliar phosphonate sprays in order to obtain data that can be used for registering phosphonate foliar sprays on avocado in South Africa. The efficacy of foliar ammonium- and potassium phosphonate sprays were evaluated when applied as four or five 0.5 g a.i. % sprays only in fall after harvest. This strategy could further reduce the risk of fruit residues, since no phosphonates will be applied when young fruit are present on trees. Furthermore, the strategy could also improve root phosphite residues since fruit are not present during application that can potentially reduce phosphite translocation to roots. The efficacy of the foliar sprays was evaluated by quantifying root phosphite and the ability of *P. cinnamomi* to colonize roots. The latter was determined indirectly using a baiting assay that determines the amounts of zoospores produced from infected avocado roots.

MATERIALS AND METHODS

Phosphonate efficacy trial layout and treatments

Seven treatments were evaluated at two orchard sites situated in different climatic regions (Letaba and Mooketsi). The Ramadiepa trial was situated in Letaba (high rainfall area, conducive to root rot), whereas the Markland trial was situated in Mooketsi (low rainfall area, less conducive to root rot). The Ramadiepa trial contained 6-year old 'Carmen' on Dusa® rootstock trees, and Markland contained 7-year old 'Maluma Hass' on Duke7® rootstock trees. The estimated tree sizes were in the order of 2.8 m high with a canopy diameter of 3.5 m, and row spacing of 7 m. The orchard trials were set in a

completely randomised block design with six replicates per treatment. Each replicate consisted of ten trees. Between the treated rows, an untreated buffer row also left. The treatments in the two trials included:

1. Untreated trees
2. Four 0.5% a.i. potassium phosphonate foliar sprays
3. Four 0.5% a.i. ammonium phosphonate foliar sprays
4. Five 0.5% a.i. potassium phosphonate foliar sprays
5. Five 0.5% a.i. ammonium phosphonate foliar sprays
6. Two trunk injections applied at the registered preventative rate (0.3 g a.i./m^2) in fall after summer flush has hardened off and in summer after the spring flush has hardened off.
7. Two trunk injections applied at the registered curative rate (0.5 g a.i./m^2) in fall after summer flush has hardened off and in summer after the spring flush has hardened off.

The potassium phosphonate product used for the foliar sprays was Fighter®, and for the ammonium phosphonate sprays Brilliant® was used. The foliar sprays were all adjusted to a pH of 7.2 using potassium hydroxide. All foliar spray treatments were only applied in fall at weekly intervals, starting five days after harvest at the Ramadiepa trial and two weeks after harvest at the Markland trial. The spray volume for all foliar treatments was calculated using the tree-row-volume (TRV) Unrath formula:

$$\text{Spray volume} = \frac{\text{tree height (2.8)} \times \text{tree canopy diameter (3.5 m)} \times 900}{\text{(row width (7m)}}$$

(McLeod *et al*., 2018). This resulted in a spray volume of approximately 1260 L/ha. All phosphonate foliar applications were made using commercial axial fan sprayers.

Evaluation of the phosphonate efficacy trials

Root sampling

Root samples were taken at the two orchards, four trees in the middle of each replicate. Both orchards did not receive phosphonates for more than one year, at which point root phosphite concentrations were very low. Root samples were taken at three time points subsequent to the trunk injection applied in fall (April 2017). The time points were 4 weeks (June 2018), 12 weeks (August 2017) and 20 weeks (Sept 2017) after the fall trunk injections. Roots were washed free from soil using tap water and air dried for ~10 min at room temperature on paper towels, and used for quantification of phosphite and *P. cinnamomi*.

Quantification of* Phytophthora cinnamomi *from roots

The roots were surface sterilised with a 70% ethanol solution for 20 sec, after which the roots were left to air dry at 22 °C for 10 min, making sure that the roots do not over dry. The surface sterilized roots from each replicate were each placed in a square plastic container along with distilled water. Citrus leaf baits were floated on the water. Baiting containers were incubated at room temperature for 72 hours. The leaf baits were plated out on the oomycete selective PARPH medium (17 g of corn meal agar in 1 L of deionized water; including pimaricin (0.005 g), ampicilin (0.125 g), rifampicin (0.01 g), PCNB (0.1 g) and hymexazol (0.05 g)) (Solel and Pinkas, 1984). The plates were incubated at 22 °C in the dark for three days, after which the plates were evaluated for hyphal growth characteristic of *P. cinnamomi*.

Root phosphite quantification

A phosphite standard curve was constructed using phosphorous acid (Sigma) and contained concentrations of 0.05, 0.1, 0.5, 1, 2.5, 5, 10 and 20 µg/ml.

Root samples were processed and extracted for phosphite as previously described by McLeod *et al*. (2018). The exception was that the water volume used for extracting phosphite was increased from 10 ml to 40 ml to improve phosphite recovery rates. The extracted phosphite samples were sent for quantification at the Central Analytical Facility (CAF) at Stellenbosch University. The samples were quantified using the Liquid Chromatography Mass Spectrometry (LC-MS/MS) method described by McLeod *et al.* (2018).

Phytotoxicity orchard trials

Two additional orchard trials were conducted, which evaluated the foliar phytotoxicity of the foliar phosphonate sprays. One trial was in the Mooketsi area and the other in the Letaba region. The treatments in the trial consisted of five foliar sprays of ammonium- or potassium phosphonate applied at 0.5% a.i. or 1% a.i., along with the untreated control. The trials were a completely randomized block design, with each of the five treatments being replicated three times. A replicate consisted of six trees, with the centre two trees being used for phytotoxicity ratings. Foliar sprays were applied as described under the "Phosphonate efficacy trial layout and treatments" section.

Phytotoxicity incidence and severity were evaluated using a rating scale for incidence and severity. The scale for rating the incidence of phytotoxicity consisted of a rating of 0 to 5, where the % of foliage affected were rated as 0 = no symptoms, 1 = 1-20%, 2 = 21-40%, 3 = 41-60%, 4 = 61-80% and 5 = 81-100%. The scale for phytotoxicity severity ratings consisted of a scale where 0 = no symptoms, 0 to 1 = mild, >1 to 2 = moderate, >2 to 3 = moderately severe, >3 to 4 = severe and >4 to 5 = extreme phytotoxic.

Statistical analyses

Analyses of variance (ANOVA) was performed on the percentage leaf discs colonized by *P. cinnamomi* and root phosphite concentrations using the GLM (General Linear Models) procedure of SAS statistical software (Version 9.4; SAS Institute Inc., Cary, USA). The Shapiro-Wilk test was performed to test for deviation from normality (Shapiro and Francia, 1972).

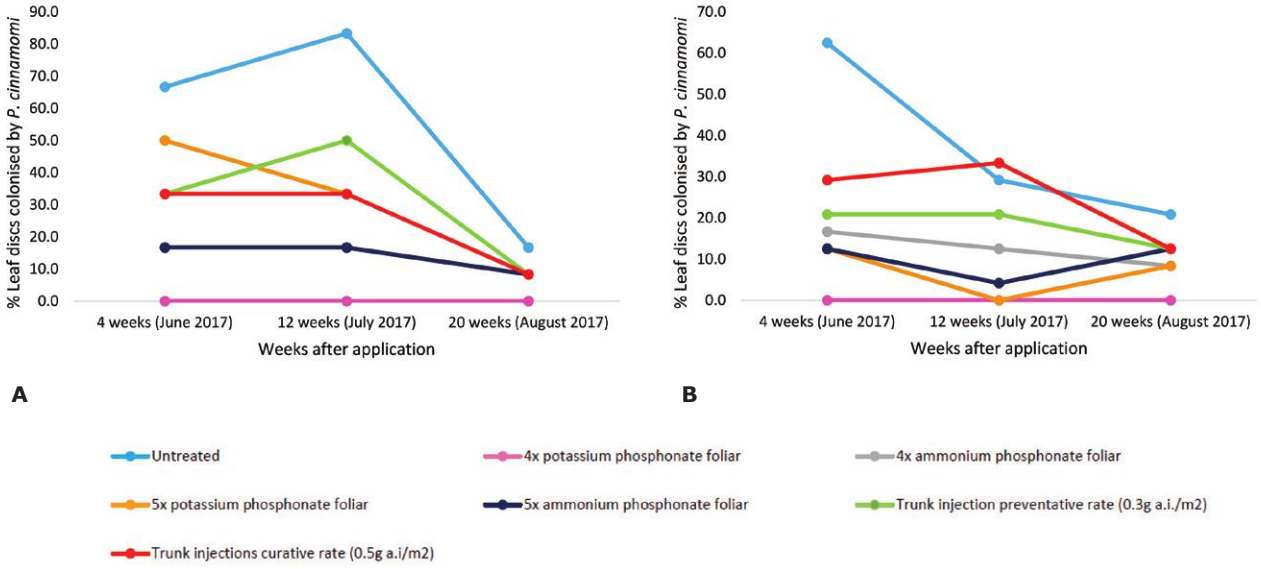


Figure 1: Effect of phosphonate treatments on the production of *Phytophthora cinnamomi* zoospores from avocado roots sampled at (A) Ramadiepa and (B) Markland. Zoospore production from roots was quantified through a root leaf disk baiting method. The average percentage of leaf disk baits colonized by *P. cinnamomi* for three time points (4-, 12- and 20 weeks) are shown.

The root phosphite data was not normally distributed and therefore a Ln (x+1) transformation was conducted to stabilize the variance and improve normality (Snedecor and Cochran, 1980). For root phosphite concentrations data, a few outliers were removed to improve normality. Fisher's least significant difference (LSD) was calculated at the 95% level to compare means for significant effects (Ott, 1998). A probability of 95% was considered significant for all significance tests.

RESULTS

Evaluation of the phosphonate efficacy trials

Quantification of Phytophthora cinnamomi *from roots*

For both trials, there were no significant differences (P > 0.05) between treatments in the percentage leaf disks colonized by *P. cinnamomi* when the 4 weeks, 12 weeks and 20 weeks after treatment sampling points were analysed separately. However, at both trials there were clear trends at the three sampling points for the untreated control roots having a higher percentage of leaf disks colonized than the roots from phosphonate treated trees (Fig. 1). The average percentage infected leaves over all three-time points were also calculated and analysed. There were no significant trial x treatment interaction (P = 0.772) for the average percentage baits infected over the three-time points. Therefore, the data of the two trials were combined for analyses. ANOVA analyses showed that there were significant differences between treatments (P = 0.0052). The control treatment had a significantly higher percentage of infected leaf baits than all of the phosphonate treatments (foliar sprays and trunk injections) (Fig. 2). Of the phosphonate treatments, the 0.3 g a.i./m^2 trunk injection treatment had the highest percentage of leaf disks infected. This was, however, not significantly higher than most of the other phosphonate treatments, except for the treatment where four potassium phosphonate foliar sprays were applied (Fig. 2).

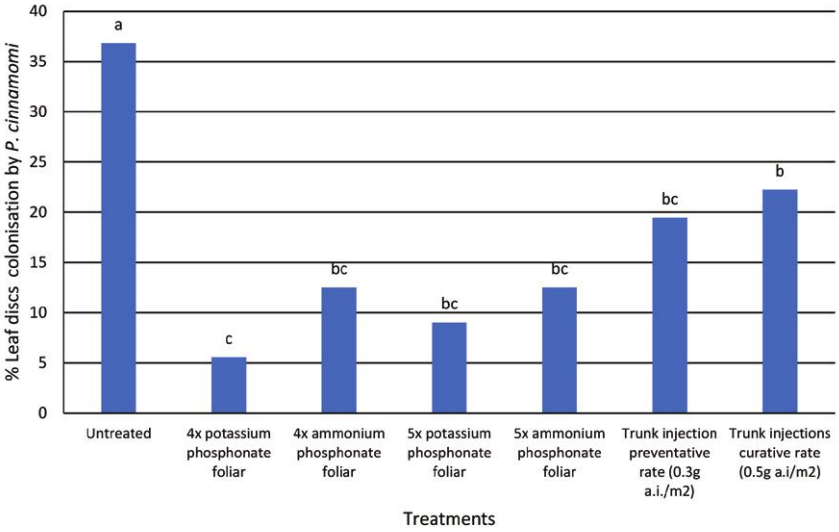


Figure 2: Effect of phosphonate treatments on the production of *Phytophthora cinnamomi* zoospores from avocado roots sampled at two orchard trials (Ramadiepa and Morgenzon). The results are shown as the average of the two trials. Zoospore production from roots was quantified through a root leaf disk baiting method. The average percentage of leaf disk baits colonized by *P. cinnamomi* for three time points are shown.

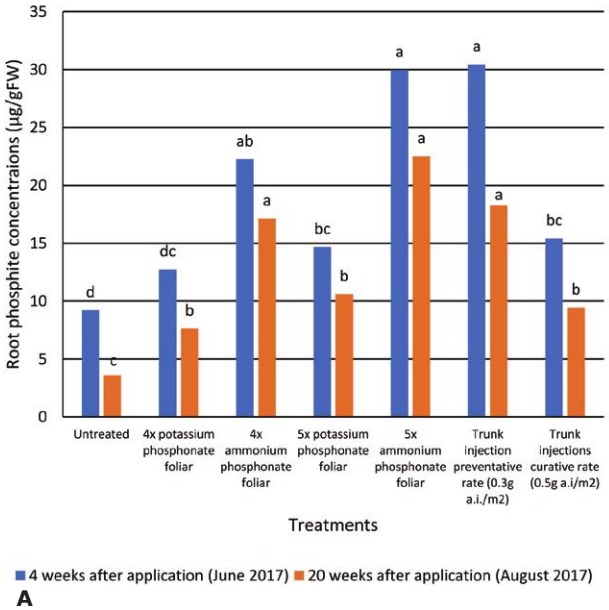
Root phosphite quantification

The quantified root phosphite concentrations were higher 4 weeks after application and subsequently declined from 4 weeks to 20 weeks after phosphonate applications were made in both trials (Fig. 3). Although the root phosphite concentrations were higher at Markland at the 4 weeks after application time point than at Ramadiepa for most treatments, the concentrations 20 weeks after application rapidly declined at Markland. This rapid decline between 4- and 20 weeks was not so evident at the Ramadiepa trial. ANOVA analyses showed that for each of the trials there were significant differences between treatments ($P < 0.0001$).

- The performance of the preventative (0.3 g a.i./m^2) and curative (0.5 g a.i./m^2) trunk injection treatments differed at the two trials. At the Markland trial, there were no significant differences in root phosphite concentrations between the two treatments at both of the time points. However, at the Ramadiepa trial the curative treatment unexpectedly yielded significantly higher root phosphite concentrations than the higher dosage preventative treatment at both time points (Fig. 3).
- In both trials, the ammonium- and potassium phosphonate foliar sprays, the four and the five sprays yielded root phosphite concentrations that were not significantly lower that the curative trunk injection treatment (Fig. 3). However, relative to the preventative trunk injection treatment at Ramadiepa, the potassium phosphonate foliar sprays (four and five sprays) yielded significantly lower root phosphite concentrations.
- The ammonium phosphonate foliar sprays often yielded significantly higher root phosphite concentrations than the corresponding number of potassium foliar phosphonate sprays (Fig. 3). At the Ramadiepa trial, the ammonium phosphonate foliar sprays consistently yielded significantly higher root phosphite concentrations than the corresponding five or four potassium phosphonate foliar sprays. However, at the Markland trial this was only true for the 20 weeks after application time point.
- Four versus five foliar sprays of potassium phosphonate yielded root phosphite concentrations that did not differ significantly from each other (Fig. 3). This was also true for the five versus four ammonium phosphonate foliar sprays. However, at 20 weeks after application there was a slight trend for the five sprays of ammonium- or potassium phosphonate yielding higher root phosphite concentrations than the corresponding four foliar sprays of each product.

Phytotoxicity orchard trials

The five foliar sprays of potassium phosphonate sprayed at 0.5% a.i. resulted in a very low incidence of phytotoxicity (<1%) and very mild phytotoxicity symptoms in both trials (Fig. 4). When the potassium phosphonate foliar sprays were sprayed at a higher dosage (1% a.i.), the incidence of phytotoxicity increased slightly (1-20%) and remained only mild in severity. The five foliar sprays of ammonium phosphonate sprayed at both dosages (0.5% and 1%) had a somewhat higher phytotoxicity incidence than the potassium phosphonate foliar sprays; incidence of 1-20% for the 0.5% a.i. sprays and 21-40% for the 1% a.i. sprays on average for the two trials. The severity of the ammonium phosphonate foliar sprays ranged from mild (0.5% a.i.) to moderate (1% a.i.).



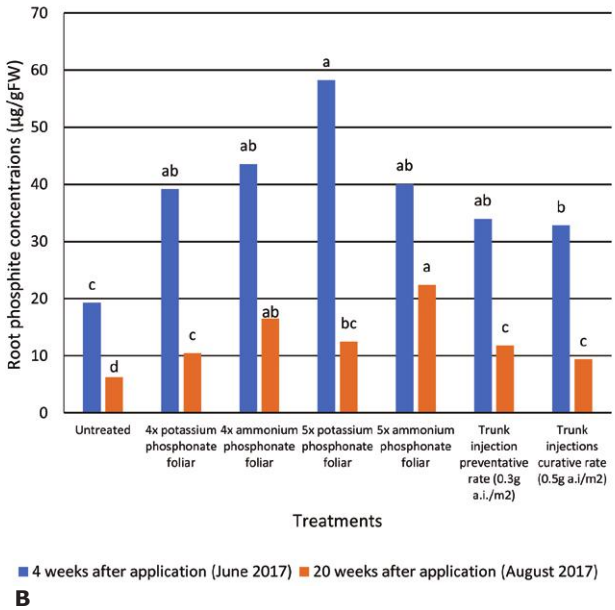


Figure 3: Root phosphite concentrations (µg/g_{FW}) in avocado trees receiving different phosphonate treatments at the (A) Ramadiepa and (B) Markland orchard trials. Root phosphite was quantified 4- and 20 weeks after the phosphonate trunk injection treatments were applied in fall. Bars of the same colour followed by the same letters do not differ significantly ($P > 0.05$).

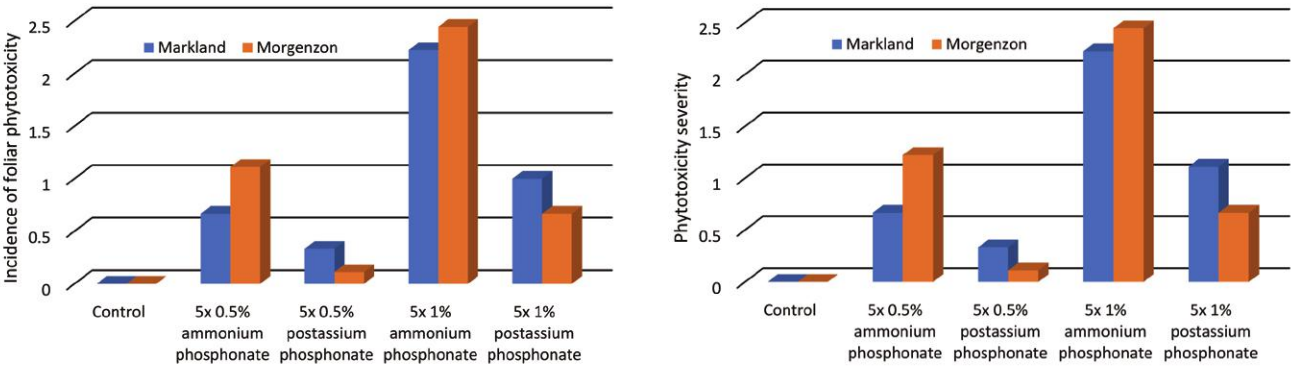


Figure 4: Evaluation of the effect of ammonium- or potassium phosphonate foliar sprays on the foliar phytotoxicity (A) incidence and (B) severity at two trials sites (Ramadiepa and Markland). Sprays were applied after harvest as five sprays at weekly intervals. The scale for the incidence of phytotoxicity consisted of a rating of 0 to 5, where the percentage of foliage affected were rated as 0 = no symptoms, 1 = 1-20%, 2 = 21-40%, 3 = 41-60%, 4 = 61-80% and 5 = 81-100%. The scale for phytotoxicity severity consisted of a scale where 0 = no symptoms, 0 to 1 = mild, >1 to 2 = moderate, >2 to 3 = moderately severe, >3 to 4 = severe and >4 to 5 = extreme phytotoxic.

DISCUSSION

The current study evaluated the potential of ammonium- and potassium phosphonate foliar sprays as an alternative to trunk injections. The efficacy of the foliar sprays was evaluated based on (i) root phosphite concentrations measured 4- and 20 weeks after application and (ii) *P. cinnamomi* root infections at 4-, 12- and 20 weeks after application. The results indicated that foliar sprays applied as four or five weekly sprays after harvest in fall were as effective as the registered split application (fall and summer) of the curative phosphonate trunk injection treatment.

It was notable that at the Markland trial, there was a substantial decrease in root phosphite from 4 weeks after application to 20 weeks after application. The rate of root phosphite decline between the two-time points was much lower at Ramadiepa. The rapid decline at Markland might be due to faster root growth in this region that has a warmer climate than the Ramadiepa trial. Due to the decline in root phosphite concentrations observed in both trials, it will be important to determine whether only fall foliar applications will be sufficient in both production regions.

Based on root phosphite concentrations, the foliar sprays, four as well as five sprays, were as effective as the curative trunk injection treatment. There were some differences in the root phosphite concentrations achieved with the ammonium- versus potassium phosphonate foliar spray treatments. The ammonium foliar phosphonates sprays, the four and five sprays, yielded significantly higher root phosphite concentrations at 20 weeks after application than the corresponding number of potassium phosphonate foliar sprays in both trials. A similar finding has been reported previously by McLeod *et al.* (2018). However, the ammonium phosphonate treatments resulted in slightly higher phytotoxicity than the corresponding potassium phosphonate treatments.

Although the ammonium phosphonate foliar sprays yielded higher root phosphite concentrations than the potassium phosphonate foliar sprays, these treatments did not differ significantly in reducing *P. cinnamomi* activity in roots. All the phosphonate treatments, including the trunk injections, resulted in a significant reduction in *P. cinnamomi* activity relative to the untreated control treatment. Furthermore, the ammonium- and potassium foliar sprays, either as four or five sprays, were as effective as the two trunk injection treatments (curative and preventative treatment) at reducing *P. cinnamomi* activity. The method used, i.e. leaf disk baiting for assessing *P. cinnamomi* activity in roots, is likely an indication of (i) the amount of *P. cinnamomi* present in roots and (ii) the potential of roots to produce new inoculum of *P. cinnamomi* once environmental conditions become favourable in the soil. Both of these aspects will be important in suppressing disease development and maintain tree health. The fact that the roots from phosphonate treated trees still yielded viable *P. cinnamomi* concentrations is a clear indication that phosphonates only suppresses the pathogen, but does not kill the pathogen in roots. It is therefore important to use an integrated strategy that includes the use of mulches or other organic materials, careful irrigation scheduling to avoid over irrigation, planting on ridges, using tolerant rootstocks and optimal inorganic nutrient applications (Wolstenholme and Sheard, 2010). The fact that phosphite containing tree roots can still produce zoospores was also reported by Wilkinson *et al.* (2001) for native Australian plant species. The current study is one of the first studies showing that root baiting with leaf disks can be used as an estimate to determine *Phytophthora* zoospore production from roots. Rollins *et al.* (2016) also reported that leaf baiting, targeting *Phytophthora ramorum,* is a very effective and sensitive technique for the semi-quantitative determination of zoospores present in stream water.

The two trials from the current study will be evaluated further since it will be important to determine the root phosphite concentrations and extent of *P. cinnamomi* root colonization at longer time spans, as

well as fruit residues. This will provide an indication as to whether the strategy of applying foliar phosphonates only in fall, and not in summer after the spring flush has hardened off, is a good approach for reducing fruit residues, and maintaining effective root rot control.

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