Evaluating phosphite concentrations in avocado tree roots following phosphonate applications – Preliminary report

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ABSTRACT

Phosphonates, which breaks down into phosphite in plants, are regularly used for the management of avocado root rot in South Africa. The first aim of the study was to determine in two orchard trials the effect of ammonium- and potassium phosphonate foliar sprays on root phosphite concentrations, in comparison to phosphonate trunk injections. The second aim was to survey healthy and declining trees in nine orchards in order to determine whether trees differing in health status differ in their ability to transport phosphite to roots 3 weeks after the application of phosphonate trunk injections. For the first aim, all phosphonate applications were made in the fall (March) following hardening off of the summer flush. This showed that three 0.5% ammonium- or potassium foliar sprays yielded similar root phosphite concentrations than the currently registered curative trunk injection dosage of 0.5 g a.i./m^2. Interestingly, a trunk injection treatment applied at a double dosage (1 g a.i./m^2) for most sampling points did not result in significantly higher root phosphite concentrations than the 0.5 g a.i./m^2 trunk injection dosage. Ammonium phosphonate foliar sprays applied at a 1% rate most often resulted in significantly higher root phosphite concentrations than the registered trunk injection (0.5 g a.i./m^2). However, this treatment resulted in severe leaf burn in one trial. The 1% potassium phosphonate foliar spray resulted in significantly higher root phosphite concentrations than the registered trunk injection in only one of the two trials. Evaluation of the root phosphite concentrations in roots of declining and healthy looking trees showed unexpectedly that the declining trees in five of the orchards contained higher root phosphite concentrations than healthy trees.

INTRODUCTION

In South Africa, avocado root rot caused by *Phytophthora cinnamomi* is controlled by most growers using potassium phosphonate trunk injections. The latter is also currently the only registered application method being used. Although alkyl phosphonate is also registered for trunk and foliar applications, these applications are not often used by growers due to cost implications. In addition to these two phosphonate salts, ammonium phosphonate is also registered on some crops for *Phytophthora* control in South Africa, but not on avocado.

Due to the fact that phosphonates are highly mobile in plants, foliar sprays are a feasible application method for a preventative root rot control strategy. Alternatives to trunk injection applications are needed since trunk injections are becoming increasingly costly. In Australia, 0.5% a.i. potassium phosphonate foliar sprays are used in a preventative control strategy on bearing avocado trees. Australian growers apply between three to six sprays per season, based on the level of root phosphite detected in roots (personal communication, WA Whiley, Sunshine Horticultural Services Pty Ltd; Thomas, 2001, 2008). It is important to note that foliar sprays are not effective when used in a curative manner, since diseased trees do not have enough foliage to take up foliar sprays.

In plants, the concentration of phosphite (also known as phosphonic acid), the breakdown product of phosphonates in plant tissue, is important for the suppression of oomycetes. Oomycetes are a group of fungal-like organisms to which *Phytophthora cinnamomi* also belongs to. In the model system of Arabidopsis and *Hyaloperonospora arabidopsidis* it has been shown, using mutant plants and gene expression studies, that at low phosphite concentrations a host plant defense mechanism is involved in pathogen suppression, whereas at higher phosphite concentrations a direct toxic effect contributes to pathogen suppression (Massoud *et al.,* 2012). A similar mode of action has also been proposed for phosphonates by Jackson *et al.* (2000) for the suppression of *P. cinnamomi* in *Eucalyptus marginata.* Therefore, measuring phosphite concentrations in plants can be indicative of pathogen suppression. Unfortunately, very little is known about the exact mode of action of phosphonates in suppressing *P. cinnamomi* in avocado, and the concentrations required for pathogen suppression. However, some progress has been made in the Australian avocado industry where root phosphite quantifications has been evaluated since the early 1980s. Long-term monitoring of root phosphite concentrations in diseased and healthy trees has resulted in the use of a 20-40 $\mu g/g_{\text{fresh weight (FW)}}$ concentration as the critical root phosphite concentration required in trees 3-4 weeks after injection (Whiley *et al.*, 2001; Giblin *et al.*, 2005). More recently, this value has been increased to 80-100 ug/g_{FW} based on information on the decline of root phosphite concentrations over time and the presence of *P. cinnamomi* isolates that are less tolerant to phosphite (personal communication, Elizabeth Dann, Queensland Alliance for Agriculture and Food Innovation, University of Queensland). Unfortunately, the method used for root phosphite concentration quantification in Australia belongs to SGS laboratories and is not available to researchers. Therefore, at Stellenbosch University, a root phosphite quantification method was developed for avocado roots (Ma, 2016). However, it is unknown whether this quantification method is comparable to that of SGS Australia.

The first aim of the current study, funded by SAAGA and ZZ2, was to evaluate the effect of different dosages of foliar phosphonate sprays and trunk injections on root phosphite concentrations. The second aim was to evaluate root phosphite concentrations in diseased and healthy roots 3 weeks after application to determine if a specific root phosphite concentration is associated with healthy versus declining trees.

MATERIALS AND METHODS

Orchard phosphonate application trials

Avocado orchard trials were conducted at two sites (Ramadiepa and Markland). Both orchards sites contain Maluma-Hass on Duke 7 trees, which have a 7 m row width spacing and 3.5 m within row tree spacing. The trees have a height of 2.5 to 2.8 m and a canopy diameter of 3.5 m. The one orchard site at Ramadiepa was located in a more tropical climate near Tzaneen, whereas the other trial at Markland was located in a region with a drier climate in Mooketsi.

The trial design in both orchards consisted of each treatment being replicated six times, in a completely randomised block design. Each replicate consisted of six trees, with roots being sampled for phosphite concentrations from the center four trees. Root samples were taken approximately 4, 12, 20 and 32 weeks after the last phosphonate foliar applications were made. The root phosphite concentration in feeder roots in each replicate sample was determined using a LC/MS-MS method (Ma, 2016).

The treatments, which were all applied in fall after the summer flush hardened off (23 March 2016), in both trials were:

1. Untreated control
2. 3 weekly foliar potassium phosphonate sprays (0.5%) applied at 7-14 day intervals
3. 3 weekly foliar ammonium phosphonate sprays (0.5%) applied at 7-14 day intervals
4. 3 weekly foliar potassium phosphonate sprays (1%) applied at 7-14 day intervals
5. 3 weekly foliar ammonium phosphonate sprays (1%) applied at 7-14 day intervals
6. One potassium phosphonate trunk injection at 0.5 g a.i./m^2 (registered rate for curative treatment in South Africa)
7. One potassium phosphonate trunk injection at 1 g a.i./m^2 .

Trunk injections were applied according to registered label recommendations, except that for the 1 g a.i./m^2 trunk injection treatment, the phosphonate dosage in syringes were double that of the 0.5 g a.i./m^2 injections. All foliar sprays were applied with commercial axial-fan sprayers that were calibrated to deliver a high spray volume according to the Unrath tree-row-volume (TRV) model. The model is very useful for determining spray volumes since it is based on the assumption that each row of orchard trees consists of a wall of foliage, where the amount of pesticide that is required is related to the volume of the foliage within the wall. The pesticide rate per hectare is calculated from the labelled rate of the chemical/100 L and the volume of foliage per hectare (Unrath *et al.*, 1986). The Unrath formula that was used was: (tree height x tree diameter x 900)/row width. This resulted in a spray volume of approximately 1125 to 1260 L/ha. All foliar spray solutions were adjusted to pH 7.2 using potassium hydroxide to prevent foliar burn.

Survey of phosphite root concentrations in healthy and diseased orchards

Growers, with the assistance of SAAGA technical staff, were asked to send root samples obtained from orchards containing healthy looking trees and trees showing symptoms of Phytophthora root rot decline. Within each orchard, approximately 500 ml feeder roots were sampled from five healthy trees and five symptomatic trees. The roots were sampled prior to injection, and again three weeks after injection. The roots from each tree was analysed for phosphite concentration as previously described (Ma, 2016).

RESULTS

Orchard phosphonate application trials

The application of the phosphonate treatments resulted in an increase in root phosphite concentration for all treatments after application, which subsequently declined from July to December in the Ramadiepa trial and from August to December in the

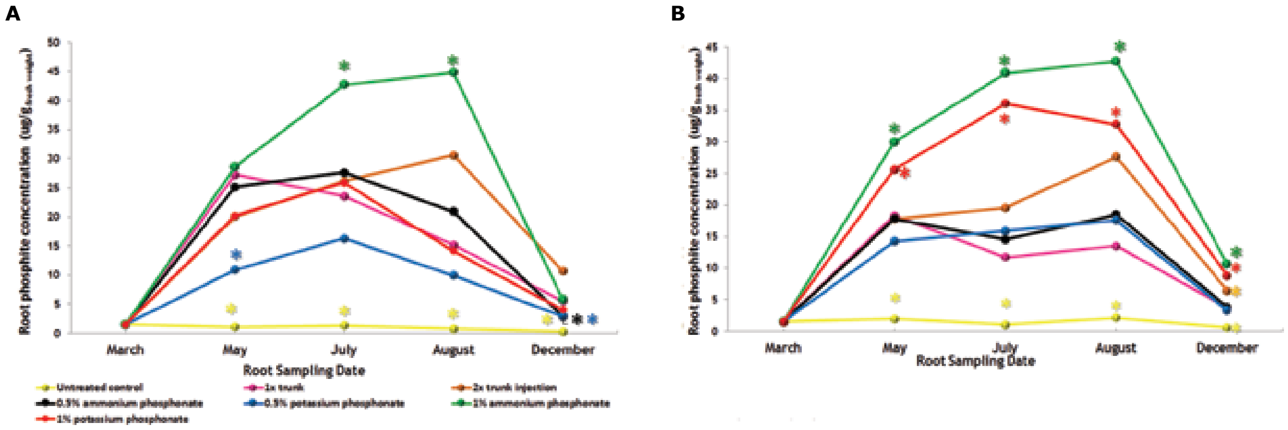

Figure 1. Root phosphite concentrations in avocado trees treated with different dosages of potassium- or ammonium phosphonate that were applied as trunk injections and foliar sprays (0.5% or 1%) in two orchard trials situated in (A) a more tropical area (Ramadiepa) and (B) a drier climate (Markland). Three foliar phosphonate sprays were applied starting 21 to 23 March 2016 at 7 to 14 day intervals, at which time trunk injections (0.5 g a.i./m^2 [1x trunk] or 1 g a.i./m^2 [2x trunk]) were also applied. Root samples were taken and analysed for phosphite concentration from March 2016 through to December 2016. The average root phosphite concentration from six replicates per treatment is shown. The sampling points of treatments that had significantly higher or lower root phosphite concentrations than the registered 0.5% trunk injection are indicated by a "*".

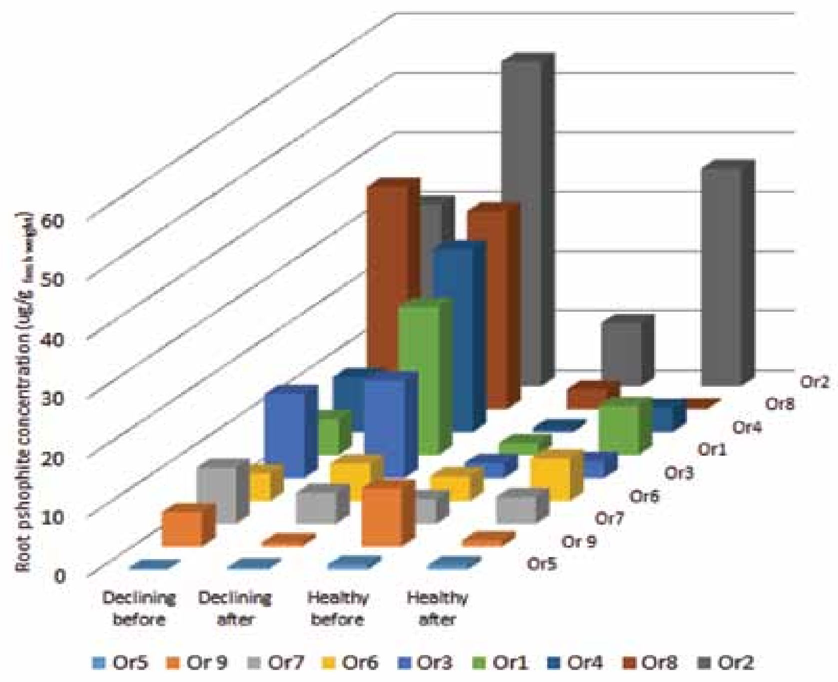

Figure 2. Root phosphite concentrations in healthy avocado trees and in trees that showed symptoms of Phytophthora root rot decline, before phosphonate trunk injections and three weeks after phosphonate trunk injections were applied. Each bar is the average the root phosphite concentration of five trees.

Markland trial (Fig. 1). The application of 0.5% foliar ammonium or potassium phosphonate sprays resulted in root phosphite concentrations that did not differ significantly from the registered trunk injection, except for the May time point from the Ramadiepa trial. The trunk injection applied at a double dosage of 1 g a.i./m^2 did not result in significantly higher root phosphite concentrations than the registered trunk injections, except for the last sampling point in December at Markland. The 1% ammonium phosphonate foliar sprays resulted in significantly higher root phosphite concentrations than the registered trunk injections at most sampling points in both trials. The 1% potassium phosphonate foliar sprays resulted in significantly higher root phosphite concentrations in only one of the two trials. The root phosphite concentrations four weeks after application of the registered trunk injection treatment and the 0.5% ammonium foliar sprays were 20-25 ug/g $_{FW}$ at Ramadiepa and 17-28 ug/g $_{FW}$ at Markland.

Survey of phosphite root concentrations in healthy and diseased orchards

The survey of root phosphite concentrations in nine orchards three weeks after injection mostly resulted in very low root phosphite concentrations irrespective of the health status of trees (Fig. 2). Of the healthy trees, only orchard 2 had root phosphite concentrations that were comparable (36 ug/g $_{FW}$) to those achieved in our orchard trials. Unexpectedly the declining trees contained higher root phosphite concentrations than the healthy trees in five of the orchards (Or1, Or2, Or3, Or4, and Or8). Furthermore, the declining trees in some of these orchards (Or2, Or3, Or7 and Or8) had relative high residual root phosphite concentrations (14-37 ug/g $_{FW}$) prior to injection.

DISCUSSION

Monitoring root phosphite concentrations was effective in comparing different phosphonate application methods and dosages. The potential of foliar sprays for replacing trunk injections was evident using this approach in two orchard trials. Based on root phosphite concentrations, three 0.5% sprays of potassium- or ammonium phosphonate applied in fall after the summer flush hardened off, were as effective as the currently registered 0.5 g a.i./m^2 trunk injection treatment. This finding is similar to the results from two other orchard trials that were conducted in 2015 (McLeod *et al.,* 2016). The 1% ammonium phosphonate sprays in both trials resulted in significantly higher root phosphite concentrations than the registered trunk injection. However, this application rate is not recommended due to the risk of phytotoxicity. The two orchard trials are still being evaluated further for root phosphite concentrations, since one additional foliar spray was applied in summer (November) after the spring flush hardened off for the 0.5% ammonium- and potassium phosphonate treatments as well as the 0.5 g a.i./m^2 trunk injection treatment. Reapplications were not made for the 1% foliar spray treatments and the 1 g a.i./m^2 trunk injection treatment. Fruit residues and yield data will be obtained for both trials, to also determine treatment effects on these parameters.

The current trials evaluated two different trunk injection dosages, including the registered curative dosage of 0.5 g a.i./m^2 and a much higher dosage of 1 g a.i./m^2. The higher dosage was included to determine if this application rate might result in prolonged root phosphite concentrations, which could eliminate the requirement for reapplication in summer after the spring flush has hardened off. Furthermore, in Australia a much higher injection rate of 1.2 g a.i./m^2 is registered than in South Africa (personal communication, Elizabeth Dann), which suggests that either our application rate is too low, or the Australian application rate is too high. South Africa's registered trunk injection dosage is based on the work of Darvas *et al*. (1984), who reported that a trunk injection application rate of 0.4 g a.i./m^2 of phosetyl-Al provided excellent curative control of Phytophthora root rot on fully grown avocado trees. Based on the results of the current trials, the 0.5 g a.i./ m^2 dosage is sufficient and potentially as effective as a 1 g a.i./m^2 dosage, since a doubling in the applied phosphonate concentration did not result in significantly higher root phosphite concentrations at most of the sampling points, except for the last sampling time in one of the trials. The reason for this is unknown, but it could be that avocado trees can only take up a limited amount of phosphonates that are applied as a single dosage.

The finding that roots of declining avocado trees in general contained higher root phosphite concentrations than healthy trees was surprising, since phosphite concentrations in plants have been reported as being positively correlated with disease suppression (Massoud *et al*., 2012). The reason for this could be that the declining trees were perhaps not specifically affected by Phytophthora root rot, but that an abiotic cause could be involved and therefore the health status of the trees is not due to root rot. Unfortunately, the presence of *P. cinnamomi* in declining trees were not tested. It is also likely that declining trees have a reduced root system, which results in the accumulation of high phosphite concentrations in a relative small root volume. The lack of growth of roots due to biotic or abiotic factors could also result in a build-up of phosphite over time in the roots, which is supported by the relative high residual root phosphite levels in declining trees in four of the orchards prior to phosphonate trunk injections. Lastly, the root samples that were received did not always consist of feeder roots, but often also contained thicker roots. The latter could have also had an effect on the measured root phosphite concentrations, since only feeder roots should be used in root phosphite analyses.

Future studies will continue evaluating in orchard trials the effect of phosphonate foliar sprays on root phosphite concentrations. The trials will be aimed specifically at determining if increasing the number of foliar sprays applied only in fall, after the summer flush has hardened off, can yield sustained root phosphite concentrations for one year until the next fall. This is important, since this would allow for the elimination of applications made in summer (Nov./Dec.) after the spring flush has hardened off. Although the label information on registered phosphonate trunk injection products recommend application during this time frame, as also suggested by Darvas *et al*. (1984), this application time frame is problematic. This is due to the fact that during this application time, small fruits on trees serve as a sink for phosphonates, resulting in high phosphite (phosphonic acid) fruit residues that are highly likely to exceed the maximum residue levels set by the European Union for our exported fruit.

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