Investigating phosphonic acid residues in late hanging fruit and in tree roots from trees that received one versus two annual trunk injections

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ABSTRACT

The first aim of the study was to investigate whether applying phosphonates to late hanging fruit resulted in increased fruit residues. A replicated trial was conducted in the Letaba and Mooketsi regions. In the Letaba trial, late hanging fruit harvested 21 days after the last foliar sprays (potassium- and ammonium phosphonate) were applied, had significantly higher fosetyl-Al fruit residues compared to fruit that were not late hanging. In the Mooketsi trial, the same strong trend was observed although it was not significant. All of the foliar sprays (potassium and ammonium) in the Letaba trial applied to late hanging fruit resulted in exceedances of the maximum residue level (MRL) 21 days post-application, but not for the at harvest fruit. In the Mooketsi trial, the ammonium phosphonate sprays resulted in an exceedance of the MRL in late hanging fruit 21 days after the last sprays were applied, but not potassium phosphonate sprays. Fruit residues in fruit harvested at the normal harvest date did not exceed the MRL in the Mooketsi trial. Phosphonate trunk injections applied to trees bearing late hanging fruit had less of a negative effect on fruit residues, since these did not result in exceedances of the MRL in both trials. The trunk injections, applied to trees containing late hanging fruit, however, also increased the risk of exceeding the MRL. The second aim of the study was to determine the effect of phosphonate trunk injections applied once or twice a year on root phosphonic acid (synonym of phosphite) concentrations over one to two seasons at different time points in three orchard trials. In one orchard, phosphonic acid root concentrations were very similar between the one and two annual trunk injection treatments, not differing by more than 7 mg/kg_{FW}. In the other two orchard trials, root phosphonic acid concentrations for the two and one trunk injection treatments did not differ by more than 9 mg/kg_{FW} for almost all of the investigated time points. The exception was for one time point in each of the trials where the two trunk injection treatments yielded root phosphonic acid concentrations that were approximately 19 mg/kg_{FW} higher than the one trunk injection treatment. In all three orchards, root phosphonic acid concentrations for the trunk injection treatments (one and two injections) were only below 25 mg/kg_{FW} (concentrations ranging from 17 and 20 mg/kg_{FW}) for the time points (February and October) just before new injections were conducted.

INTRODUCTION

Phosphonate fungicides are used extensively in South Africa to manage Phytophthora root rot (PRR) in avocado. Until 2013, fruit residues resulting from phosphonate applications were not a concern, since a maximum residue level (MRL) was not enforced worldwide. However, in 2013, the European Union (EU), which is South Africa's largest export market, started enforcing an MRL of 50 mg/kg for phosphonates on avocado. The fruit residues resulting from potassium phosphonate applications, which is the phosphonate formulation registered in South Africa, are expressed as fosetyl-Al (sum of fosetyl plus phosphonic acid and their salts expressed as fosetyl) (Commission regulation [EU] 2016/1003). This is somewhat confusing, since potassium phosphonate only breaks down to phosphonic acid. Fosetyl is only found in the commercial formulation of Aliette®, which is an alkyl phosphonate. The matter has been evaluated by the European Food Safety Authority (EFSA). Therefore, a new MRL definition accommodating potassium phosphonate (phosphonic acid and their salts, expressed as phosphonic acid) has been proposed by the peer review group (EFSA *et al.*, 2018). This, however, is not yet enforced.

Limited information is available on how fruit residues are affected when phosphonate applications are applied onto mature fruit. Whiley (2001) reported that the application of one 0.5% a.i. or 1% a.i. potassium phosphonate foliar spray to fruit at commercial maturity (approximately 25% dry matter) only had a minor effect on fruit residues. Fruit residues resulting from these treatments 1 to 14 days after the foliar spray were applied, were low (2.70 to 4.30 mg/kg) and similar for the 1% and 0.5% sprays (Whiley, 2001). Phosphonate trunk injection of trees bearing mature fruit has also been reported to yield minimal fruit residues (unpublished results within Whiley, 2001). This is likely due to the fact that mature fruit is not a strong sink for phosphonic acid. The first aim of the current study was to determine whether phosphonate applications (foliar sprays and trunk injections) applied to mature fruit that were left to hang on trees after harvest (late hanging fruit) resulted in an increase in fruit residues.

Registered potassium phosphonate products on avocado specify that two trunk injections should be applied annually. One injection is made in summer (after the spring foliar flush hardened off), whereas the other is applied in fall (usually after harvest when the summer foliar flush hardened off). These applications, however, can sometimes result in exceedances of the EU MRL in fruit. The trunk injection applied in summer is most likely contributing the most to fruit residues, since small fruit are present on trees that act as a strong sink for phosphonic acid (McLeod *et al.*, 2018). Therefore, several growers have considered reducing the number of trunk injections to one annual injection, which is applied after harvest. This approach may affect PRR management in the long-term. The second aim of the current study was thus to investigate the effect of one versus two trunk injections on phosphonic acid (synonym phosphite) root residues. Root phosphonic acid concentrations can be indicative of the efficacy of phosphonates against PRR (McLeod *et al.*, 2018; Masikane *et al.*, 2020).

MATERIALS AND METHODS

Effect of phosphonate applications to mature fruit on fruit residues

A replicated trial was conducted in the Mooketsi and Letaba production regions. The trials had a completely randomized block design, containing six treatments (Fig. 1). Each treatment was replicated six times. The Letaba trial contained 'Hass' trees grafted onto Duke 7, and the Mooketsi trial 'Maluma-Hass' also grafted onto Duke 7. All foliar phosphonate treatments were applied in autumn after harvest in 2018 and 2019 as four weekly 0.5 a.i. or 0.25% a.i. sprays as previously described (Masikane *et al.*, 2020). Two products, an ammonium phosphonate (Brilliant®) and potassium phosphonate (Fighter®) were used for the foliar spray treatments. Trunk injections were applied using a potassium phosphonate product (Avoguard®) in autumn and in summer (after the spring flush hardened off) in 2018 and in 2019 according to label recommendations.

Fruit harvested after receiving the autumn applications in 2019, resembling late hanging fruit, were assessed for fosetyl-Al residues by an accredited laboratory (Hearshaw and Kinnes Analytical Laboratory [Pty] Ltd, Tokai, South Africa). Fruit residues were also assessed in fruit that were harvested at the normal harvest time in 2019.

Effect of one versus two annual trunk injections on phosphonic acid root concentrations

Three trials were initiated in 2018 in three orchards (BT, ET and FT) without PRR symptoms. Two of the orchards were situated in the Letaba region (BT and ET) and one in the Mookesti region (FT) in South Africa.

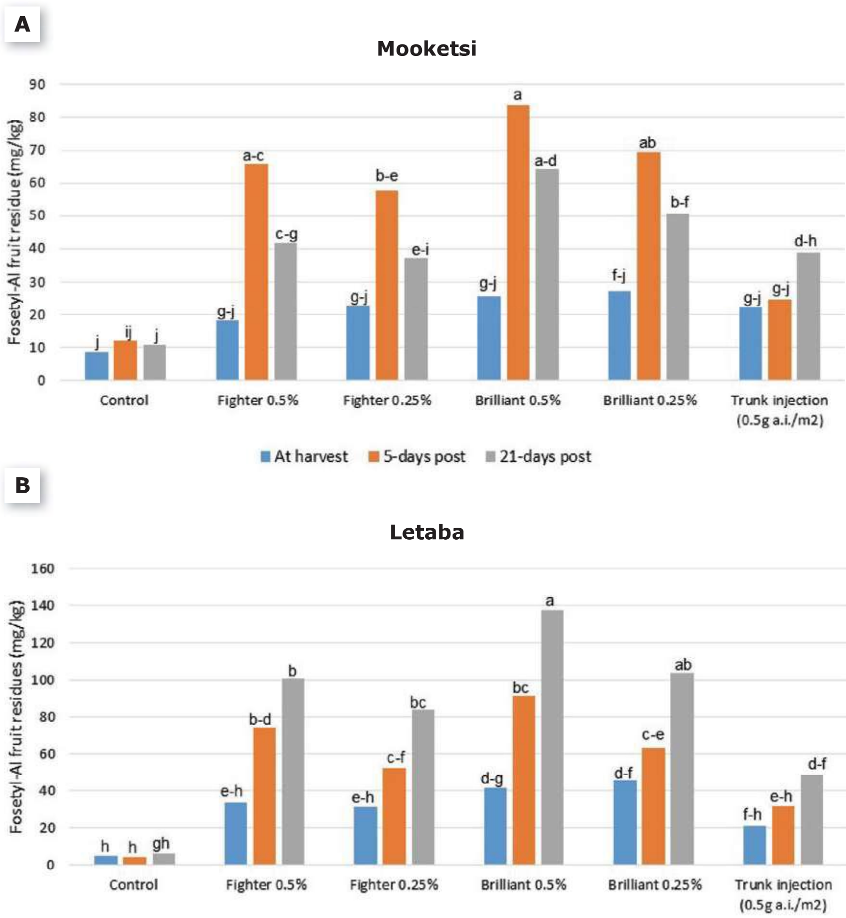

Figure 1: Effect of phosphonate applications on fruit residues (fosetyl-Al) in late hanging fruit in two trials located in (A) Mooketsi and (B) Letaba

Three treatments were evaluated in each of the orchards: (i) untreated control, (ii) 1x trunk injection and (iii) 2x trunk injections. Each treatment contained eight trees that were randomly selected within a one hectare area. For two of the orchards (ET and FT), the 1x trunk injection treatment was applied to trees in April 2018 and April 2019. For the 2x trunk injections treatment in these orchards, trees were injected in November 2017, April 2018, November 2018 and April 2019. For the BT orchard, trunk injections were only applied starting in 2018. Each trunk injection was applied at a preventative dosage of 0.3 g a.i./m^2 (Avoguard® 500 SL; Nulandis, Kempton Park, South Africa) in 2017 and 2018, but at the curative dosage (0.5 g a.i./m^2) in 2019.

Tree roots were sampled four times (May and October 2018, and February and July 2019) for two of the orchards (ET and FT), whereas for the BT orchard roots were only sampled in February and July 2019 (Fig. 2). Root phosphonic acid was extracted and quantified in tree roots as previously described (Masikane *et al.*, 2020).

Statistical analyses

The late hanging fruit trial residue data were analysed using Statistica 12 (Dell Software). Analyses of variance was conducted, followed by post hoc testing (Students least significant difference test) at the 95% confidence level. Root phosphonic acid concentrations from the trunk injection trials have not yet been analysed statistically.

RESULTS

Effect of phosphonate application to mature fruit on fruit residues

In the Mooketsi trial, there was a trend for the 21-days post application fruit residues (fosetyl-Al) from foliar sprays being lower than the 5-days post application residues (Fig. 1A). This, however, was not significant for any of the treatments. Fruit residues were significantly higher for the 21-days post application treatment than for the at harvest fruit for the Brilliant foliar sprays (0.5% and 0.25% a.i). All of the fruit residues resulting from the Fighter foliar sprays were below the MRL only for the at harvest and 21-days post application treatments, but not the 5-days post application treatment. However, for the Brilliant foliar sprays (0.5% and 0.25% a.i.) the MRL was exceeded for the 5- and 21-days treatments but not the at harvest treatment. Although there was a trend for the trunk injection fruit residues to increase from at harvest to 21-days post application, this was not significant (Fig. 1A).

In the Letaba trial, there was a trend that fosetyl-Al fruit residues increased from 5- to 21-days post application of phosphonates to late hanging fruit for all phosphonate treatments (Fig. 1B). This however, was only significant for the Brilliant sprays (0.5% and 0.25% a.i.). All foliar sprays resulted in significantly higher fruit residues for the 21-days postharvest treatment than for the at harvest treatment. The trunk injection treatment did not result in significantly higher fruit residues for the late hanging fruit, in comparison to residues of fruit at the normal harvest date. Fruit residues 21-days post application of all phosphonate foliar sprays all exceeded the MRL, whereas the injection treatment almost (48.28 mg/kg) exceeded the MRL (Fig. 1B).

Effect of one versus two annual trunk injections on phosphonic acid root concentrations

Phosphonic acid root concentrations in the BT orchard were very similar between the one and two annual trunk injection treatments, with the one trunk injection having a slightly higher (7 mg/kg) root phosphonic acid concentration than the two trunk injection treatment only for the July 2019 time point (Fig. 2A). In the ET orchard, the two versus one trunk injections treatment did not differ by more than 9 mg/kg root phosphonic acid root concentration for almost all of the time points. The exception was for the July 2019 time point where the two trunk injection treatment yielded a root phosphonic acid concentration that was 18 mg/kg higher than the one trunk injection treatment (Fig. 2B). In the FT orchard, a similar result was obtained than for the ET orchard, except that the October 2018 time points was the time point that differed by more than 9 mg/kg_{FW} root phosphonic acid concentration; the two trunk injections had a 19 mg/kg_{FW} higher root phosphonic acid content than the one trunk injection (Fig. 2C). In all three orchards, root phosphonic acid concentrations for the trunk injection treatments were sometimes below 25 mg/kg_{FW} for the time points just before new applications were made (February and October); these concentrations ranged between 17 and 20 mg/kg_{FW} (Fig. 2).

DISCUSSION

The effect of phosphonate foliar sprays on fruit residues when applied to late hanging fruit differed between the Mooketsi and Letaba trial. In the Letaba trial, fruit residues tended to increase from 5- to 21-days, whereas in the Mooketsi trial they decreased. The increase in fruit residues in the Letaba trial at 21-days post application suggests that phosphonic acid is translocated to mature fruit. This was unexpected, since previous reports indicated that mature fruit is not a sink for phosphonic acid (Whiley, 2001). It is unknown why phosphonic acid translocation was more evident in mature fruit in the Letaba trial than in the Mooketsi trial, and why residues were higher in Letaba than in the Mooketsi trial. Residues in late hanging fruit receiving foliar sprays consistently exceeded the MRL in the Letaba trial, and only sometimes in Mooketsi trial. The fact that translocation of phosphonic acid to mature fruit can occur under certain circumstances when foliar sprays are applied, has resulted in a recommendation that this practice should not be applied. If late hanging fruit are present when phosphonates are applied, it might be better to rather apply trunk injections. In the current study, fruit residues seemed less

IoT and irrigation scheduling

The Internet of Things (IoT) has become a buzzword in agriculture over the past few years, promising to connect all things hardware to all things digital. What really matters however, is to ensure that there is strategic focus on what is being connected and what data is being produced, so that the information has practical relevance, is user friendly and can be implemented to make a meaningful difference on the farm.

James Moore

IRRICHECK IS SA'S largest independent irrigation scheduling consulting firm with irrigation scheduling consultants throughout SA and SADC. We are independent of the soil moisture probe manufacturers; and supply, install and maintain ***all the trusted brands***, and provide our own proprietary irrigation scheduling software, IrriCheck Pulse™.

At IrriCheck, we have taken a strategically prudent decision to connect sensors relating to and focused on irrigation scheduling to the IrriCheck Pulse™ irrigation scheduling platform, making decisions around irrigation management and water use even more precise.

Sensors include:

- **Weather stations:** Provides us with reliable and accurate farm-specific forecast data, as well as ETo (Reference Evapotranspiration) data. This is in effect the demand placed on the crop from the atmospheric conditions, and will directly impact the daily water requirement for any given crop.
- **In-line flow meters:** This allows us to accurately and timeously measure water applied per tree, in litres or cubes per hectare.
- **In-line EC meters:** Placed into pivots, micro or dripper lines, these sensors send us data on water quality and/or fertigation dosage. Measuring in mS/cm, this can help identify if water quality is poor and/or if fertiliser concentrations are not sufficient or worse, too high, negatively affecting your roots and soils.
- **Canopy sensors:** This sensor allows us to accurately and timeously receive data measuring VPD (Vapour Pressure Deficit), which is an indication of the stress being applied to the crop through the demand on transpiration, primarily as a result of temperature, humidity, wind and sunlight radiation.
- **Sap flow meters:** Installed into the tree, these sensors send us data on the rate of sap flow under different environmental conditions and importantly, in relation to data from other sensors, such as VPD and the loss of soil moisture in millimetres, derived from the soil moisture probes. Together with the probe, this data provides very accurate information on actual ETc (Evapotranspiration of the Crop) and factors influencing this.

- **Dendrometers:** Placed onto fruit, this sensor sends us accurate and timeous data on the growth of the fruit. This, again, used alongside data on environmental conditions, VPD, sap flow and soil moisture data from the probe, provides us with a holistic view and valuable, interdependent information directly affecting fruit growth, which can then be manipulated by means of irrigation scheduling to achieve optimum size and quality.

These IoT sensors link directly with telemetry units at the probe site and send data, which interfaces directly with the IrriCheck Pulse™ irrigation scheduling platform available on your smartphone, tablet or desktop computer. Data is then interpreted, providing meaningful information, which enables our irrigation schedulers and our clients to make well-informed changes to irrigation schedules to achieve real results that are scientifically based.

affected when trunk injections were applied to trees bearing late hanging fruit than when foliar sprays were used.

In general, in all three of the orchard trials where one versus two trunk injections were compared, root phosphonic acid concentrations did not differ much. The exception was for one of the time points in two of the orchards. Finding similar root phosphonic acid concentrations for the one- and two trunk injection treatments was unexpected, since the two annual trunk injection treatment in effect received double the dosage of phosphonic acid than the one annual trunk injection. This phenomenon might be explained by the fact that the two annual trunk injection treatment is applied when trees contain small fruit, unlike the one trunk injection treatment. Thus, some root phosphonic acid might be lost to fruit in the two annual trunk injection treatment. Fruit residues were not measured in the trials to determine whether this did indeed occur. Except for the time points just before new injections were applied, trees receiving one- or two annual trunk injections all had root phosphonic acid concentrations above 25 mg/kg$_{FW}$. In Australia, root phosphonic acid concentrations of 25 to 40 mg/kg$_{FW}$ are considered sufficient for disease control and are used as a guide for applying phosphonates commercially (personal communication, G. Thomas, GLT Horticultural Service, Frederickton, NSW, Australia). Whether these critical root phosphonic acid concentrations are also applicable to South Africa is unknown, but it may differ since rootstocks used in the two countries differ in tolerance and our root phosphonic acid quantification method may be different. The injection trials from the current study will be continued for the 2020/21 season by measuring root phoshonic acid concentrations and *P. cinnamomi* root quantities. Tree ratings were also conducted at the start of the trials and will be conducted again at the end of the trials to determine if the treatments had an effect on canopy health.

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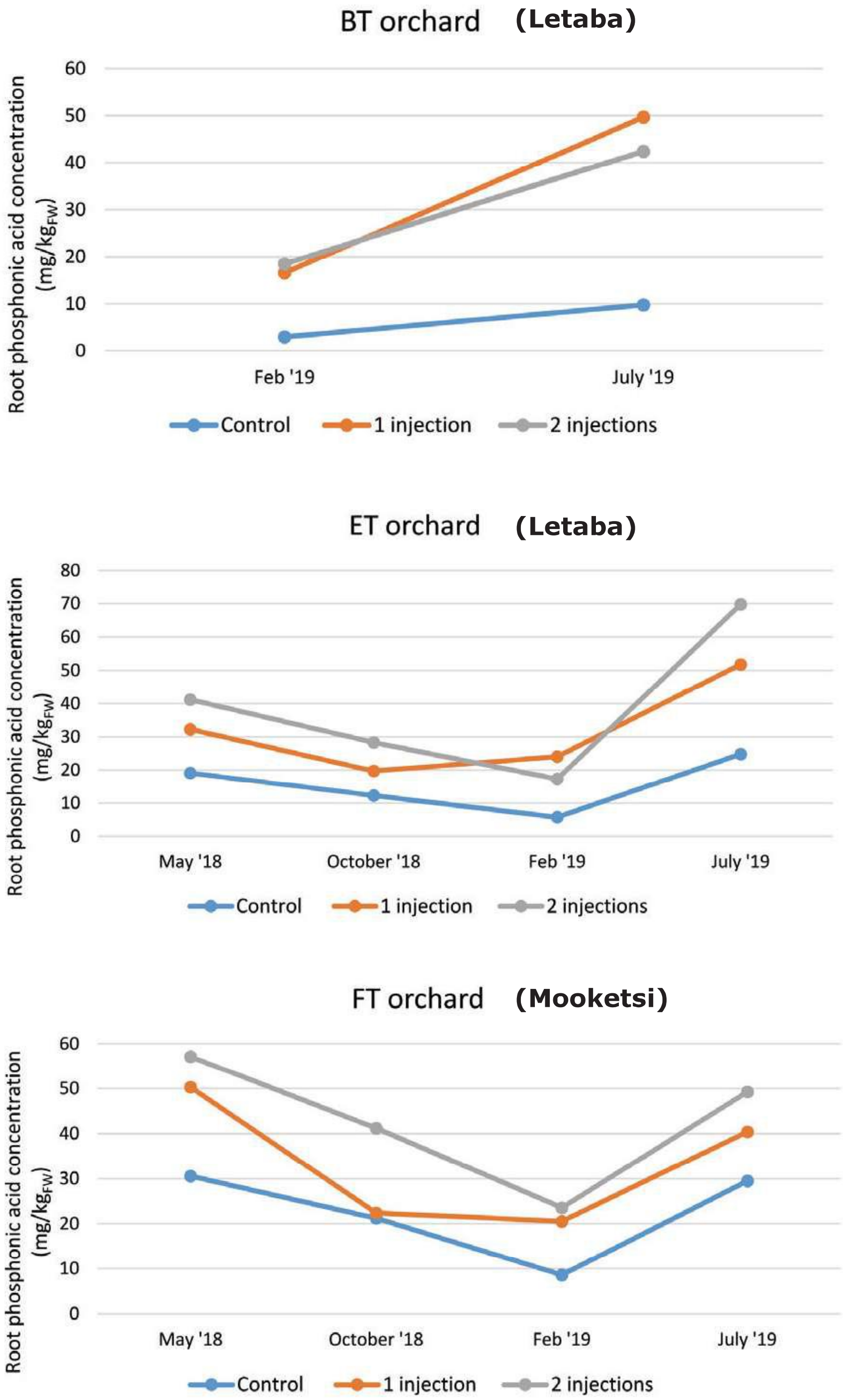

Figure 2: Effect of phosphonate trunk injections on root phosphonic acid concentrations over several sampling months in three avocado orchards

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