Phosphonate application effects on growth of citrus seedlings and root infections by *Phytophthora nicotianae*

JAN VAN NIEKERK[1,2]

ELAINE BASSON[1]

CHARMAINE OLIVIER[1]

[1]*Citrus Research International (Pty) Ltd., P.O. Box 28, Nelspruit, 1200, South Africa*

[2]*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa*

**Corresponding author, e-mail: janvn@cri.co.za*

Contamination of citrus nurseries by soil-borne pathogens, especially *Phytophthora nicotianae* and *Phytophthora citrophthora*, is a big risk to the sustainability of a nursery. Therefore, the presence of these two pathogens in nurseries should be prevented at all cost, especially since infected nursery trees are often the source of infection in orchards (Ippolito et al. 2004).

Phosphonate sprays are used in citrus production as part of a preventative program for the control of root rot and brown rot of fruit caused by the abovementioned *Phytophthora* spp. (Oren and Yogev 2002). Phosphonates via foliar application are readily absorbed by the leaves, after which they are translocated via the phloem to fruit or new roots (Ouimette and Coffey 1990). It affects the pathogen directly at the host-pathogen interaction level, while also activating the plant's own defence mechanisms (Fenn and Coffey 1984; 1985; Afek and Sztejnberg 1989; Smillie et al. 1989).

The application of phosphonate to container-grown citrus trees was evaluated previously and the results of these studies indicated that foliar or drench applications reduced the level of root infections by *P. nicotianae*, while increasing seedling size (Walker 1988; Oren and Yogev 2002). However, previous studies on citrus seedlings only evaluated the effect of a single phosphonate application.

Foliar applications of phosphonates are employed by some nurserymen in South African citrus nurseries as part of their preventative *Phytophthora* management program. However, some questions remain with regard to which type to use, frequency of application, method of application and whether applications prevent the tree roots from becoming infected by *Phytophthora* spp. The aim of this study was to compare the efficacy of different phosphonates to reduce *Phytophthora* root infections, optimise the application regime and identify the best application method to use. The effect of these applications on *P. nicotianae* root infections was furthermore evaluated.

Materials and methods

Citrus nursery seedlings

Carrizo citrange seedlings were transplanted into 5 ℓ plastic planting bags containing a 50:50 mixture of steam-sterilised river sand and composted pine bark. Seedlings were grown for a period of six months before the trial commenced.

Phosphonate application dosages, methods and regimes

Ammonium phosphite (Brilliant, Arysta LifeScience, Umhlanga, South Africa), potassium phosphite (Fighter, Agchem, Pretoria, South Africa) or potassium phosphonate (Phytex 200 SL, Horticura cc, Gezina, South Africa) were applied to the seedlings as either a foliar spray or root drench. Dosages were applied according to label recommendations for mature bearing citrus trees with application set at six week intervals. Foliar applications on seedlings were sprayed to just before run-off. Drench application involved drenching seedling bags with 540 ml solution per 5 ℓ bag. The foliar sprays and drench applications were applied according to three different application regimes. The regimes were as follows: Regime 1 involved only winter applications (May, July and August 2019), Regime 2 was a summer only application (December 2018, January and March 2019) while Regime 3 combined summer and winter applications. Two control sets of seedlings were included: one set of seedlings was only inoculated, whereas another set received neither inoculation nor chemical treatment.

Phytophthora nicotianae inoculum preparation and inoculation

Phytophthora nicotianae inoculum was prepared by inoculating 1 ℓ Erlenmeyer flasks containing a 10% V8 broth with 20 large media plugs cut from 9-day-old *P. nicotianae* cultures. Following inoculation, the flasks were placed on a reciprocal shaker at 120 rpm for 14 days. Mycelium was then separated from the media plugs and blended – seedlings were ultimately inoculated with ± 0.5 - 1 g *P. nicotianae* mycelium, suspended in 50 ml distilled water, twice weekly for four weeks. The first inoculation occurred from November to December 2018, followed one week later by the first chemical application (summer treatment); the second inoculation was in May 2019 and was followed one week later by the second chemical application (winter treatment).

Trial evaluation

Two weeks after the final applications, the trial was evaluated destructively (September 2019). Tree height, root mass, shoot mass and tree mass were determined for each treatment combination. *Phytophthora nicotianae* infestation levels in roots were determined by doing isolations from the roots. All recorded data were statistically analysed.

Results

Plant measurements

The ANOVA done with the different plant measurements indicated a significant chemical × application type interaction for seedling mass ($P = 0.0001$) and shoot length ($P = 0.0073$). Ammonium phosphite foliar sprays resulted in seedlings with a mean mass of 126.60 g that was significantly better than the mean of the seedlings receiving drench applications of this chemical (105.10 g). Both these means were significantly better than the mass of the untreated, inoculated control (99.93 g; Table 1). Foliar sprays with potassium phosphite resulted in seedlings with a mean mass of 118.83 g, statistically better than the mean mass of the drench application seedlings (98.94 g) that was not different from the control (Table 1). The statistically best mean seedling mass (128.43 g) was achieved through potassium phosphonate drench applications. This mean was also significantly better than the mean seedling mass of 114.29 g, resulting from foliar applications of the same chemical. Although not always significant, applications of the different chemicals resulted in seedlings with higher seedling mass means than the untreated, inoculated control (Table 1).

The seedling mass data also revealed in the ANOVA a significant ($P = 0.0229$) chemical × application regime interaction. Regimes 1 and 2 resulted in statistically similar means of 124.07 g and 123.16 g, respectively (Table 2). This was statistically better than the mass of regime 3 (100.33 g) and also the untreated control (99.93 g). In the case of potassium phosphite, there was no significant difference in the mean seedling mass resulting from the three application regimes; the means were between 102.44 g and 113.44 g (Table 2). Furthermore, none of these means were statistically better than the mean of the untreated control. For potassium phosphonate, regime 3

Table 1. Mean plant biomass and shoot length observed for Carrizo citrange seedlings to which ammonium phosphite, potassium phosphite or potassium phosphonate was applied as either a drench or foliar spray application.

Chemical	Application type	Mean plant biomass (g)	Mean shoot length (mm)
Ammonium phosphite	Drench	105,10 c-e[1]	1482,38 ab
	Foliar spray	126,60 b	1610,60 ab
Potassium phosphite	Drench	98,94 e	1424,33 bc
	Foliar spray	118,83 a-c	1665,73 a
Potassium phosphonate	Drench	128,43 a	1651,45 a
	Foliar spray	114,29 b-d	1517,84 ab
Untreated control	-	99,93 de	1290,61 c
P-value		0,0001	0,007

[1] Means followed by the same letter are not significantly different at a 95% confidence level ($P = 0.05$).

performed the best with a mean seedling mass of 127.86 g. This was similar to the mean of regime 2 at 123.42 g. Both these means were significantly better than the untreated control. However, they were not different from the mean of regime 1 (112.78 g) that was also not different from the untreated control (Table 2).

Phytophthora infected root pieces

Results from the isolations from the seedling roots indicated a significant ($P < 0.0001$) chemical effect (Table 3). The mean percentage infected root pieces for the three different chemicals were between 0.08% and 0.89% (Table 3). These were all significantly better than in the untreated control where the mean percentage infected root pieces was 16.07%.

Discussion

Foliar applications of phosphonates are used in citrus production to prevent root and collar or brown rot and therefore forms part of the preventative management program in many citrus nurseries. Determined in the current study were the efficacy of three different phosphonate products applied according to one of three regimes, using two different methods of application. This was done by evaluating the effect of the applications on seedling growth and root infections by *P. nicotianae*.

Results indicated that regardless of the type of phosphonate chemical applied or the method of application, these applications generally led to Carrizo citrange seedlings that were significantly bigger than the untreated control seedlings. Furthermore, foliar applications mostly led to better results compared to the soil drench applications, although these differences were not always significant (Table 1).

Different application regimes were evaluated to determine optimal application of phosphonates to containerised citrus seedlings, which had not been studied previously. Application regime results varied between the different phosphonate chemical treatments with no discernible trend, although mean plant biomass was consistently, if not significantly, improved when compared to the untreated control (Table 2). In the case of potassium phosphite and potassium phosphonate, the best results were observed with either summer only, or winter plus summer applications (combined treatment). Ammonium phosphite performed the best with either winter only or summer only applications. Although, both potassium phosphite and phosphonate treatments had improved mean plant biomass for summer applications compared to winter applications, ammonium phosphite had the exact opposite result. Summer applications seem to be important as the results indicated that alone, or in combination with winter applications, it gave the best results overall.

Ultimately, this study was aimed at determining the optimal method of application and application regime of phosphonates for the preventative management of *P. nicotianae* root infections. It was seen that application regime or method did not play a significant role in the efficacy of the phosphonate

Table 2. Mean plant biomass measured for Carrizo citrange seedlings to which ammonium phosphite, potassium phosphite or potassium phosphonate was applied according to three different application regimes.

Chemical	Application regime[2]	Mean plant biomass (g)
Ammonium phosphite	1	124,07 ab1
	2	123,16 ab
	3	100,33 c
Potassium phosphite	1	102,44 c
	2	113,44 a-c
	3	110,78 bc
Potassium phosphonate	1	112,78 a-c
	2	123,42 ab
	3	127,86 a
Untreated control	-	99,93 c
P-value	0,0229	

[1] Means followed by the same letter are not significantly different at a 95% confidence level (P = 0.05).

[2]Regime 1 involved a winter only a pplication, Regime 2 was a summer only application and Regime 3 involved a combined summer and winter application.

applications in reducing root infections. Applications of all three phosphonate products were found to significantly reduce root infections caused by *P. nicotianae* (Table 3).

From results obtained, phosphonate applications to citrus seedlings lead to bigger seedlings with reduced *Phytophthora* root infection. Summer, foliar applications alone or in combination with winter applications were found to be the most optimal method and timing of application. Therefore, it can be recommended to citrus nurseries that preventative phosphonate applications will be the most effective when done as foliar applications during summer.

Table 3. Mean percentage Phytophthora nicotianae infected root pieces recorded from inoculated Carrizo citrange seedlings to which ammonium phosphite, potassium phosphite or potassium phosphonate was applied.

Treatment	Mean % *Phytophthora* infested root pieces
Ammonium phosphite	0,30 a[1]
Potassium phosphite	0,89 a
Potassium phosphonate	0,08 a
Untreated control	16,07 b
P-value	< 0,0001

[1] *Means followed by the same letter are not significantly different at a 95% confidence level (P = 0.05).*

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