UPTAKE, DISTRIBUTION AND EFFECTS OF PHOSPHITE ON FUSARIUM OXYSPORUM IN PALMS

By

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To my family and friends

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LIST OF ABBREVIATIONS

DI	Deionized Water
KOH	Potassium Hydroxide
PDA	Potato Dextrose Agar

Abstract of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Master of Science

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Phosphite compounds have been shown to reduce plant disease, however the mode of action for phosphite is complex and not fully understood. Knowledge of phosphite distribution and dynamics in plants is essential to determine which diseases it may control and to determine the optimum timing for fungicide treatment. Phosphite has controlled Phytophthora bud rot in palms. Its effects on Fusarium wilt in palms are unknown. Our objectives for this study were: 1) to determine the uptake and distribution of phosphite in trunk-injected coconut palms over time, 2) to determine if *Fusarium oxysporum* f. sp*. palmarum* is sensitive to phosphite *in vitro*, and 3) compare the results with greenhouse trials on *Washingtonia robusta*. This study shows that phosphite has a long persistence and is not evenly distributed in coconut palm, but it is concentrated in the spear leaf and young and old leaf rachises. Two *Fusarium oxysporum* isolates showed sensitivity to phosphite *in vitro*. Inoculated *W. robusta* seedlings with a foliar phosphite concentration of about 350 $\mu g \cdot g^{1}$ had a lower mortality rate than those with lower foliar concentrations of phosphite.

CHAPTER 1
INTRODUCTION

Phosphites (syn. phosphonates) ($H_2PO_3^-$; HPO_3^{2-}) are alkali metal salts of phosphorous acid (H_3PO_3), a reduced form of phosphate (PO_4^{3-}). Phosphite has a higher degree of solubility and mobility, as it has one less oxygen molecule than phosphate. This characteristic gives phosphite the ability to rapidly cross the membranes of leaves or roots. It is also considered an ambimobile nutrient, which means it has both xylem and phloem mobility (Guest and Grant, 1991). Other benefits of phosphite include: persistence in plant tissue, controlled release of phosphorus during crop growth, enhanced plant and root development, improved plant health, multiple action sites as a fungicide, and low environmental toxicity.

Among the advantages of phosphite compounds, the most widely recognized property is its extremely effective control of plant diseases caused by *Phytophthora* spp. (Fenn and Coffey, 1984; Forster et al., 1998; Grant et al., 1992; Guest and Grant, 1991; Guest et al., 1995; Jackson et al., 2000; Jee et al., 2002; Smillie et al., 1989). Phosphite compounds have been shown to reduce disease (*Phytophthora infestans*, *Fusarium solani* and *Rhizoctonia solani*) severity in potato seed tubers and foliage(Lobato et al., 2008; Lobato et al., 2010), and also have a positive effect on hot and sweet pepper against *Phytophthora capsici* (Fernando Cesar Sala, 2004).

However, the action mode of phosphite is complex and not fully understood. Knowledge of phosphite distribution and dynamics in plant tissues is essential to determine the optimum timing for fungicide treatment (Bezuidenhout et al., 1987). A study on the dynamics and distribution of phosphite in avocado trees treated with phosetyl- Al indicates that the phosphite concentrations were higher in branches than in

the roots or leaves (Bezuidenhout et al., 1987). Its concentration in the branches and root samples taken from the Fuerte avocado trees reached its peak a month after trunk injection of phosetyl-Al. However, in mature leaves, the phosphite peak was broader. As a result, old branches stored about 50% of the phosphite, the roots around 30%, and the last 20% was in young braches, leaves and leaf stems. The conclusion from this study was that phosphite was not evenly distributed among different plant organs. Nothing is known about the dynamics and distribution of phosphite in palms.

The objectives for this study include: 1) determine the uptake and distribution of phosphite in trunk-injected coconut palms over time, 2) determine if *Fusarium oxysporum* f. sp*. palmarum* is sensitive to phosphite *in vitro*, and 3) compare the *in vitro* results with greenhouse trials on *Washingtonia robusta* inoculated with *Fusarium oxysporum* f. sp*. palmarum* to determine if phosphite had direct or indirect effects on this pathogen.

CHAPTER 2
LITERATURE REVIEW

Phosphite

Palms are beautiful ornamental plants and are economically important. As monocots, single-stemmed palms are relatively easy to grow but are also easily killed because they have a single apical meristem. Once the meristem is damaged, the palm usually dies. This damage could be caused by infectious diseases or physiological disorders. For example, palm bud rot, is a common disease caused by *Phytophthora palmivora* (Oomycetes). The first symptom of a bud rot caused by this pathogen is the discoloration and wilting of the spear leaf, the youngest and unopened leaf. The next youngest leaves may also appear discolored and wilted. Eventually, all leaves become desiccated, turn brown and collapse. The leaf bases often have distinct brown or necrotic areas. Fosetyl-Al, which has phosphite as its active ingredient, is the recommended fungicide for juvenile palms for control of bud rot caused by *Phytophthora* (Elliott, 2009). It has been shown that phosphite is effective in controlling *Phytophthora* in coconut palms, and that stem injection is more effective than other methods (de Franqueville and Renard, 1989). In another study, optimum long-term control of *Phytophthora palmivora* was achieved in cocoa by using stem injected potassium phosphite (Guest et al., 1994).

Trunk injection of potassium phosphite showed efficient and durable control of *Phytophthora* diseases in avocado (Pegg et al., 1985). A study on the dynamics and distribution of phosphite in avocado trees treated with phosetyl- Al indicated that the phosphite concentrations were higher in branches than in the roots or leaves (Bezuidenhout et al., 1987). Its concentration in the branches and root samples taken

from the Fuerte avocado trees reached its peak a month after the injection of phosetyl-Al. However, in mature leaves, the phosphite peak was broader. As a result, old branches stored about 50% of the phosphite, the roots around 30%, and the last 20% was in young braches, leaves and leaf stems. The conclusion from this study was that phosphite was not evenly distributed among the different plant organs. Knowledge of phosphite distribution and dynamics in plants is essential to determine optimum time for fungicide treatment (Bezuidenhout et al., 1987). However, nothing is known about the dynamics and distribution of phosphite in palms.

Foliar sprays and soil drenches are two common methods of applying phosphite. Studies have shown that foliar treatment with phosphite can control *Phytophthora cinnamoni* in infected avocado trees (Pegg et al., 1985). Although there are differences among phosphite salts applied, all of them enhance efficacy in controlling *Phytophthora.* In addition, agronomic performance of infected trees, including number of leaves, photosynthetic activity, etc. improved significantly in phosphite-treated trees (Cervera et al., 2007).

In plant tissues, phosetyl-Al degrades to ethanol and phosphite, the latter is the toxophore by either activating defense mechanisms in the plant (Bompeix and Saindrenan, 1984) or by acting directly on the fungus (Fenn and Coffey, 1984). Plants cannot utilize phosphite as a phosphorus source (MacIntyre et al., 1950). However, some bacteria are capable of converting phosphite to phosphate (Malacinski and Konetzka, 1966).

Fusarium Wilt of Palms

Fusarium wilt is a common vascular disease in some species of palm. It is caused by specific formae speciales of the fungal pathogen *Fusarium oxysporum*.

When the fungus grows in the xylem of palms, xylem tissues become obstructed by the fungus causing a failure to conduct water and other nutrients throughout the trunk, stems and leaves of palms. Currently, there are four known formae speciales of *F. oxysporum* affecting palms throughout the world: *Fusarium oxysporum* f. sp. *albedinis* (*F. o. albedinis*), *Fusarium oxysporum* f. sp. *canariensis* (*F. o. canariensis*), *Fusarium oxysporum* f. sp. *elaeidis* (*F. o. elaeidis*), and *Fusarium oxysporum* f. sp. *palmarum* (*F. o. palmarum*). No matter which Fusarium wilt is affecting palms, they die within several weeks to months after the initial infection (Elliott et al., 2004; Elliott et al., 2010).

Fusarium oxysporum f. sp. *palmarum* attacks *Syagrus romanzoffiana* (queen palm) and *Washingtonia robusta* (Mexican fan palm) and it has also been reported to attack *Phoenix canariensis* (Canary Island date palm) in Florida (Elliott, 2011).

No teleomorph of *Fusarium oxysporum* (Ascomycota) has been observed in culture. On potato dextrose agar (PDA), *F. o. palmarum* colonies have pale pinkish or purple color on the upper surface. Macroconidia, formed in orange sporodochia, are three to four septate with a curved apical cell and a foot- shaped basal cell. Microconidia are unicellular and oval, elliptical or reniform in shape. Within 4 weeks after isolation, chlamydospores are produced. On PDA, some isolates produce sclerotia that are blue in color (Elliott et al., 2010).

Fusarium oxysporum is a soilborne fungus. Chlamydospores produced by this fungus can survive in plant debris and soil for years (Elliott et al., 2004). Transmission of disease caused by *Fusarium oxysporum* depends on the movement of infected trees or infested soil and is also possible through the use of contaminated pruning tools.

Once palms are affected by Fusarium wilt, there is no known effective control. Preventive chemical controls have also not been shown to be efficacious or cost effective. Currently, the control of this disease of palms is focused on prevention. An important aspect of prevention involves proper sterilization of tools between pruning different palms. Selection of non-host palm species is important, especially if there is a history of previous disease incidences. This is important because even if diseased palms have already been removed, infective spores are likely to still be contaminating the soil. Once a palm is known to be affected by Fusarium, it should be cut down and burned or otherwise removed because as long as it remains in place it provides a potential source of infection for the surrounding landscape (Elliott et al., 2004).

CHAPTER 3
MATERIALS AND METHODS

Dynamics and Distribution of Injected HPO_3^{2-}

Plant Material

Twenty trees of *Cocos nucifera* L. 'Malayan Dwarf' (Coconut palm) growing at the University of Florida Fort Lauderdale Research and Education Center, Davie, FL., were used in this study. The Coconut palms were approximately 30 years old, 8-10 m in overall height, and were fruiting at the time of this study. Twenty replicates were arranged in a completely randomized design. Coconut palms were growing in a Margate Fine Sand soil, with individual palms spaced about 6 m apart. Palms were fertilized every 3 months by broadcasting an 8N-0.9P-10K-4Mg plus micronutrients controlled release fertilizer (Nurserymen's Sure Gro, Vero Beach, FL) at a rate of 75 g m^{-2} to the entire plot.

Injection System

The Arborjet@ tree I.V. Micro-infusion system (Arborjet, Inc., Woburn, MA) was used to inject the following four treatments: deionized water (DI) only (control), 30 mL potassium phosphite in 90 mL DI, 60 mL potassium phosphite in 180 mL DI, and 90 mL potassium phosphite in 270 mL DI. The commercial source of the potassium phosphate was StarPhite@ (Loveland Products, Inc., Greeley, CO). Holes 9.5 mm in diameter and 10 cm deep were drilled approximately one meter above the ground. All of the holes were on the south side of trunk. Treatments were replicated 5 times and were arranged in a completely randomized design. Injections were performed May 17, 2012.

Sample Collection

Plant tissues were harvested at 1, 5, 10, 15, 20, and 30 weeks after application. The following plant tissues were harvested to determine phosphite dynamics in coconut: spear leaf tip, old leaf rachis base, central leaflets from an old leaf, and central leaflets from the youngest fully expanded leaf.

On February 11, 2013, forty weeks after injection of potassium phosphite, ten different plant tissues were harvested from 90 mL potassium phosphite treatment palms and numbered as: 1. spear leaf (newest unopened leaf) tip, 2. rachis base from an old leaf, 3. rachis base from the youngest fully expanded leaf, 4. distal portion of the rachis on an old leaf, 5. distal portion of the rachis of the youngest fully expanded leaf, 6. basal leaflets from an old leaf, 7. basal leaflets from the youngest fully expanded leaf, 8. distal leaflets from an old leaf, 9. basal half of the distal leaflets from an old leaf, and 10. distal half of the distal leaflets from an old leaf. These plant tissues were analyzed for determination of phosphite distribution in coconut.

Plant Analysis

Plant samples were dried at 60°C for 48 hrs and ground in a Wiley Mill to pass through a 40-mesh screen. Homogeneous powder samples (0.5g) were extracted with deionized water, shaken by Labquake shaker (Labindustries, Inc, Berkeley, CA) for 10 minutes and centrifuged for 10 minutes at 2400 rpm. The liquid supernatant was then filtered through a 0.22 µm nylon membrane filter using a vacuum pump. The filtrate was collected and refiltered through C18 SEP- PAK Cartridges. This filtrate was then brought to a final sample volume of 5 mL. Samples were kept frozen at -11°C until they could be analyzed.

Ion Chromatography

Ion chromatography is an ideal method for analyzing anions in plant materials. There is a published a method using ion chromatography to determine HPO_3^{2-} in tomato leaflets (Fenn and Coffey, 1989). Smilie and Grant (1988) described a rapid and feasible method of using ion chromatography to determine phosphite and phosphate concentrations in plant materials.

Samples were injected by hand using a 5 ml syringe into a Dionex-100 Ion Chromatograph (Model DX1-03) (Dionex Corp., Sunnyvale, CA) with integrated Anion Self-regenerating Suppressor-Ultra, an AS-17 column, eluent gradient system and were eluted with 100 mM potassium hydroxide at a flow rate of 0.56 mL min^{-1} during the retention time of 0 - 0.45 min and 7.45 - 8.15 min at 18% of 100 mM KOH, and 5.45 - 7.45 min at 100% of 100mM KOH.. Anions were detected by a conductivity detector and the data processed using Peak Net Data acquisition system (Dionex Corp., Sunnyvale, CA). Standards containing chloride, nitrite, phosphite, bromide, and nitrate were prepared daily at three dilutions.

Phosphite concentration data from coconut leaves at 40 weeks were analyzed using ANOVA in Proc GLM, with mean separation using Tukey's HSD (α=0.05) SAS (SAS Institute, Cary, N.C.) .

Effects of Phosphite on *Fusarium oxysporum In Vitro*

Isolates

Two isolates of *Fusarium oxysporum* f. sp. *palmarum* from *Washingtonia robusta* (Mexican fan palm) (PLM-249A) and from *Syagrus romanzoffiana* (Queen Palm) (PLM-140B) were used in this experiment.

Base Media

The base medium was 1/5 strength Difco potato dextrose agar (1/5 PDA). Various amounts of potassium phosphite (StarPhite, Loveland Products, Inc., Greeley, CO) were added to 1/5 PDA to obtain 0 (control), 1, 5, 10, 20, 40, or 60 $\mu g \cdot mL^{-1}$ of phosphite. StarPhite contains 28% phoshorous acid (H_3PO_3) that has been neutralized with potassium hydroxide.

Growth of Mycelium and Radial Measurements

Twenty-eight plates of PLM-249A (four replicates per level of phosphite) and 28 plates (four replicates per level of phosphite) of PLM-140B were inoculated and then placed in 28 °C incubator in darkness. Mycelial growth in each plate was determined by measuring the diameter of the colony (in two directions) per plate every 24 hours from 2 to 6 days following inoculation (Davis, et al., 1994). The experiment was conducted twice, and the data combined for analysis.

The mycelia diameter on six dates at all rates were analyzed using regression analysis and the diameters on the sixth day at all rates were analyzed using ANOVA (SAS, SAS Institute, Cary, N.C.).

Effects of Phosphite on *Fusarium Oxysporum In Vivo*

Growing Substrate

Washingtonia robusta seedlings were grown in a greenhouse at the Fort Lauderdale Research and Education Center in 1 liter containers filled with 50% pine bark, 40% sedge peat and 10% sand substrate amended with 15N-$9P_2O_5$-$12K_2O$ Osmocote (Scotts Co., Marysville, OH) controlled release fertilizer incorporated at a rate of 10.86 kg/m^3. No herbicides were used to control weeds. An overhead irrigation system provided ca. 2 cm of water daily.

Phosphite Treatment

Eight replicate plants per treatment were used for inoculation with the pathogen and 8 additional replicates were used as horticulture controls and for leaf phosphite analysis. Potassium phosphite (Starphite®) was used in this treatment with foliar sprays or soil drenches applied. The control group received only water. This experiment was conducted twice. The first trial was conducted from February to June in 2012 with 5 treatments including: control, foliar sprays (spray to run off) or soil drenches (50 mL) every 3 weeks at 0.74 $\mu g \cdot mL^{-1}$ (2.64 $mL \cdot L^{-1}$) or 1.48 $\mu g \cdot mL^{-1}$ (5.28 $mL \cdot L^{-1}$). The second trial was performed from November 2012 to March 2013 with 9 treatments including: control, foliar spray or soil drench applied only once at the 2 rates, or foliar sprays and soil drenches applied every 3 weeks at the 2 rates.

Horticulture Control

The horticulture control seedlings were harvested on May 16, 2012 for trial one and on February 22, 2013 for trial two. Recently matured leaves of *W. robusta* palms were collected for leaf analysis of phosphite concentrations 14 weeks after first treatment.

Plant Inoculation

One month after the first phosphite treatment, the small palms (5 or 6 leaves) were inoculated with a spore suspension of PLM-249A (10^6 spores ml^{-1}; 25 ml per palm) by using soil drench inoculation on February 29, 2012 for trial one and on November 23, 2012 for trial 2.

Plant Analysis

Leaf samples were dried at 60°C at 48 hrs, ground in a Wiley Mill to pass through a 40-mesh screen. The powdered 0.5 g samples were extracted with deionized

water; shaken by Labquake shaker (Labindustries, Inc., Berkeley, CA) for 10 minutes and centrifuged for 10 minutes at 2400 rpm. The liquid supernatant was then filtered through a 0.22 µm nylon membrane filter using a vacuum pump. The filtrate was collected and refiltered through C18 SEP- PAK Cartridges. This filtrate was then brought to a final sample volume of 5 mL. Samples were kept frozen at -11°C until they could be analyzed.

Plant dry weight and phosphite concentration data were analyzed using ANOVA (SAS, SAS Institute, Cary, N.C.).

CHAPTER 4
RESULTS AND DISCUSSION

Dynamics of Injected HPO_3^{2-}

The phosphite concentrations from the first sampling of the control palms which received no phosphite showed that there very little (<25 $\mu g \cdot g^{-1}$) naturally-occuring phosphite in mature coconut palms. In old leaf rachis tissue, phosphite was rapidly taken up within the first week after injection, with the phosphite content reaching a peak at 242.7 $\mu g \cdot g^{-1}$ for the 30 mL phosphite treatment, 324.58 $\mu g \cdot g^{-1}$ for the 60 mL phosphite treatment, and 677.51 $\mu g \cdot g^{-1}$ for the 90 mL phosphite treatment (Fig. 4-1). After the first week, phosphite concentrations in all three treatments decreased until around five weeks. At that time, the concentration of phosphite in the 90 mL phosphite treatment increased and reached a peak of ≈ 550 $\mu g \cdot g^{-1}$ and then decreased, instead of decreasing for the first ten weeks like the other two treatments. However at the tenth week, the 30 mL treatment concentration increased from 80 $\mu g \cdot g^{-1}$ to 180 $\mu g \cdot g^{-1}$ for 5 weeks and then, as the other treatments, phosphite concentrations in all three rates stabilized at 151 $\mu g \cdot g^{-1}$ for the 30 mL, 174 $\mu g \cdot g^{-1}$ for the 60 mL, and about 340 $\mu g \cdot g^{-1}$ for the 90mL treatments for another 20 weeks (Fig. 4-1). The increased concentration of phosphite occurred at 90 mL treatment between 5 - 10 weeks and 30 mL treatment between 10 -15 weeks with an approximate increased value of 100 $\mu g \cdot g^{-1}$ (450 - 550 $\mu g \cdot g^{-1}$ and 80 -180$\mu g \cdot g^{-1}$ for treatment 90 mL and 30 mL, respectively.) After application, phosphite is rapidly absorbed in xylem, but is also translocated within the phloem (Guest and Grant, 1991). The decreasing and increasing concentration during the first twenty weeks could possibly be caused by phosphite equilibration and mobilization. Since the rachis serves as the connection of vascular part of the palm and

the leaf blade, the mobilization of phosphite is expected. These numbers indicate that phosphite concentrations are highest at one week after injection, and after 15 weeks, phosphite concentrations in old leaf rachis tissue remains fairly stable for at least another 6 months (Fig. 4-1).

In the spear leaf, it took ten weeks to reach the phosphite concentration peak of 313 $\mu g \cdot g^{-1}$ for the 30 mL treatment, 297.03 $\mu g \cdot g^{-1}$ for the 60 mL treatment, and 831.3$\mu g \cdot g^{-1}$ for the 90 mL treatment. After 15 weeks, phosphite concentration declined slowly over time for the 90 mL treatment. This indicates that the maximum accumulation of phosphite occurred about 10 weeks after application for the spear leaf. The phosphite concentrations of these three rates were around 350 $\mu g \cdot g^{-1}$ for 90 mL treatment and around 150 - 250 $\mu g \cdot g^{-1}$ for the other two treatments (Fig. 4-2).

Unlike the spear leaf, young leaflets take up phosphite immediately after application. Phosphite concentration reached their peaks within one week after application at 374.26 $\mu g \cdot g^{-1}$ for the 30 mL treatment, 395.732 $\mu g \cdot g^{-1}$ for the 60 mL treatment, and 860.26 $\mu g \cdot g^{-1}$ for the 90 mL treatment (Fig. 4-3). During the next four weeks, phosphite concentrations decreased significantly. In young leaf leaflets, the 30 mL and 60 mL treatments were similar for all sampling dates. The 90 mL treatment had twice the phosphite concentrations compared to the other two treatments. However, those three treatments had similar concentrations after the fifth week.

A similar trend was observed in the old leaflets, although the inflection point appeared at the tenth week (Fig. 4-4). In old leaf leaflets, phosphite was also absorbed rapidly after application with concentrations at one week of 127.73 $\mu g \cdot g^{-1}$ and 279 $\mu g \cdot g^{-1}$ for the 30 mL/90 mL and 60 mL/180 mL treatments, respectively, and 504.74 $\mu g \cdot g^{-1}$ for

the 90 mL/270 mL treatment. The phosphite concentration in highest treatment (90 mL/270 mL) decrease from 500 $\mu g \cdot g^{-1}$ to less than 100 $\mu g \cdot g^{-1}$ in the first ten weeks, and then phosphite concentration from all rates stay every similar, except for the 30 mL/90 mL and 60 mL/180 mL treatments, after 5 weeks of phosphite application, the phosphite concentration in old leaflets start to be stable around 70 - 90 $\mu g \cdot g^{-1}$. Which indicate that for an effective disease control concentration, after 10 weeks these three rates provide similar efficacy.

Distribution of Injected HPO_3^{2-}

At 40 weeks after injection with 90 mL potassium phosphite, 10 different parts of the coconut palm were sampled and phosphite content was determined. The spear leaf tip contained an average of 249.45 $\mu g \cdot g^{-1}$ phosphite. For the same plant position, tissue from young leaves consistently had higher phosphite content than older leaves. For example, young leaf rachis bases had 420.22 $\mu g \cdot g^{-1}$ of phosphite compared to 321.63 $\mu g \cdot g^{-1}$ for old leaf rachis bases. The same was true for distal rachis, basal leaflet and distal leaflet tissues (Tab. 4-1). The high concentrations in leaf rachis indicate that phosphite accumulated at the position where the pathogen *Fusarium* oxysporum would infect the palm.

Phosphite content decreased gradually from basal to distal portions in both young and old leaves (Tab. 4-1). This was expected since the palms were treated via trunk injection and phosphite should be translocated through xylem to the canopy. Spear leaves maintained a fairly high concentration for at least 10 months. The higher phosphite concentration in spear leaves come from phosphite remobilization from old tissue. This also explains why phosphite showed efficacy on *Phytophthora* bud rot on

palms, since the spear leaf is where the phosphite accumulation occurred and where the pathogen first infects the palm.

Effects of Phosphite on *Fusarium Oxysporum In Vitro*

In vitro, phosphite directly inhibited PLM-249A and PLM-140B growth. As phosphite concentration increased, inhibition of phosphite on these two isolates of *F. o. palmarum* increased. PLM-140B growth was similar for 1/5 PDA with no phosphite and 1 $\mu g \cdot mL^{-1}$ phosphite. However, growth rates decreased from 10.86 mm at 5 $\mu g \cdot mL^{-1}$, 10.36 mm at10 $\mu g \cdot mL^{-1}$, 7.21 mm at 20 $\mu g \cdot mL^{-1}$, to 3.58 mm at 40 $\mu g \cdot mL^{-1}$ (Fig. 4-5).

For PLM-249A, when phosphite concentration was increased from 0 $\mu g \cdot mL^{-1}$ to 60 $\mu g \cdot mL^{-1}$, the growth of isolates decreased significantly. Compared with PLM-140B, phosphite caused greater growth suppression in PLM-249A (Fig. 4-5).

In the repeat trial, the two isolates' sensitivity to phosphite was similar. In conclusion, both isolates of *F. o. palmarum* appeared to be sensitive to phosphite *in vitro*.

Effects of Phosphite on *Fusarium Oxysporum In Vivo*

In the first trial, the lowest mortality of *W. robusta* was achieved by application of 0.74 $\mu g \cdot mL^{-1}$ soil drench, which led to a phosphite concentration of 209.6 $\mu g \cdot g^{-1}$ However, there is no significant difference with control, which contained a phosphite concentration of 187.92 $\mu g \cdot g^{-1}$(Tab. 4-2). The relatively high concentration of phosphite in untreated *W. robusta* is unexpected as there is no natural phosphite in plant tissue documented. This requires a further determination of phosphite in *W. robusta* leaf tissue.

In the second trial, phosphite concentrations in the youngest fully-expanded leaves of *W. robusta* seedlings treated every 3 weeks with 0.74 or 1.48 $\mu g \cdot mL^{-1}$

averaged 330.49 $\mu g \cdot g^{-1}$ and 377.7 $\mu g \cdot g^{-1}$, respectively. The mortality rate of these seedlings averaged 6.25% which is significantly lower than the other treatments (Tab. 4-3). The average mortality was calculated based on similar foliar phosphite concentrations because this was a better way to show the relationship between phosphite sensitivity and concentration (Tab. 4-3). This suggested the possibility that a lower concentration of a foliar spray applied every 3 weeks was a feasible application of potassium phosphite from this experiment. More treatments and repeats need be added, and inoculation of *F. o. palmarum* for consistency in mortality needs to be improved.

The dry weights from the seedlings surviving after inoculation were analyzed, but there was no significant relationship between seedling dry weight and phosphite content or phosphite treatment. This suggests that phosphite does not quantitatively reduce growth inhibition caused by Fusarium wilt.

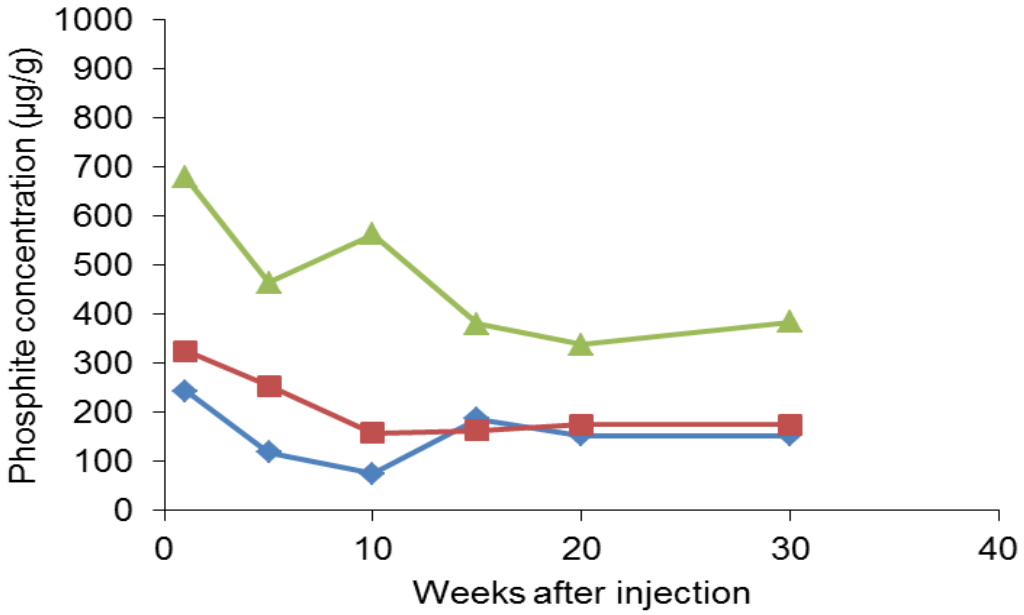

Figure 4-1. Phosphite concentration (µg·g^{-1}) in old rachis of coconut through 30 weeks following trunk injection with 3 rates of potassium phosphite: 30mL potassium phosphite in 90mL DI (◇), 60mL potassium phosphite in 180mL DI(■), and 90mL potassium phosphite in 270mL DI (▲).

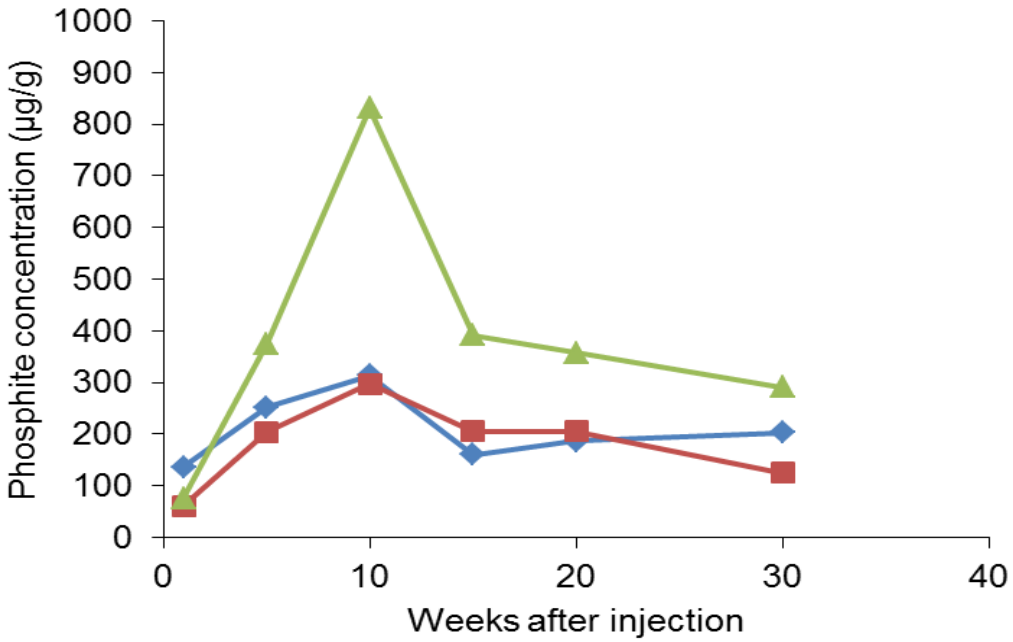

Figure 4-2. Phosphite concentration ($\mu g \cdot g^{-1}$) in spear of coconut through 30 weeks following trunk injection with 3 rates of potassium phosphite: 30mL potassium phosphite in 90mL DI (◇), 60mL potassium phosphite in 180mL DI(■), and 90mL potassium phosphite in 270mL DI (▲).

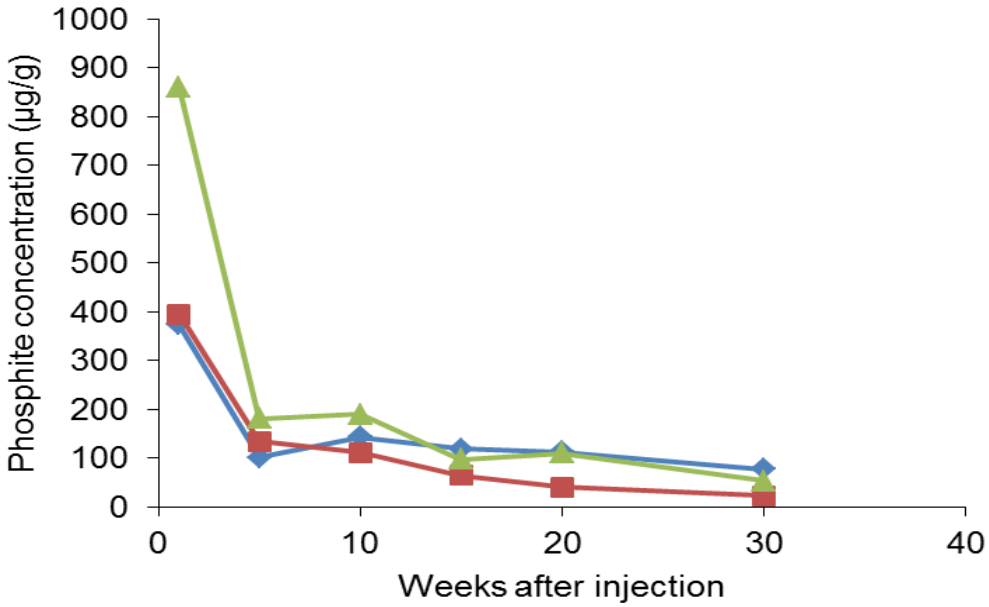

Figure 4-3. Phosphite concentration ($\mu g \cdot g^{-1}$) in young leaflets of coconut through 30 weeks following trunk injection with 3 rates of potassium phosphite: 30mL potassium phosphite in 90mL DI (◇), 60mL potassium phosphite in 180mL DI(■), and 90mL potassium phosphite in 270mL DI (▲).

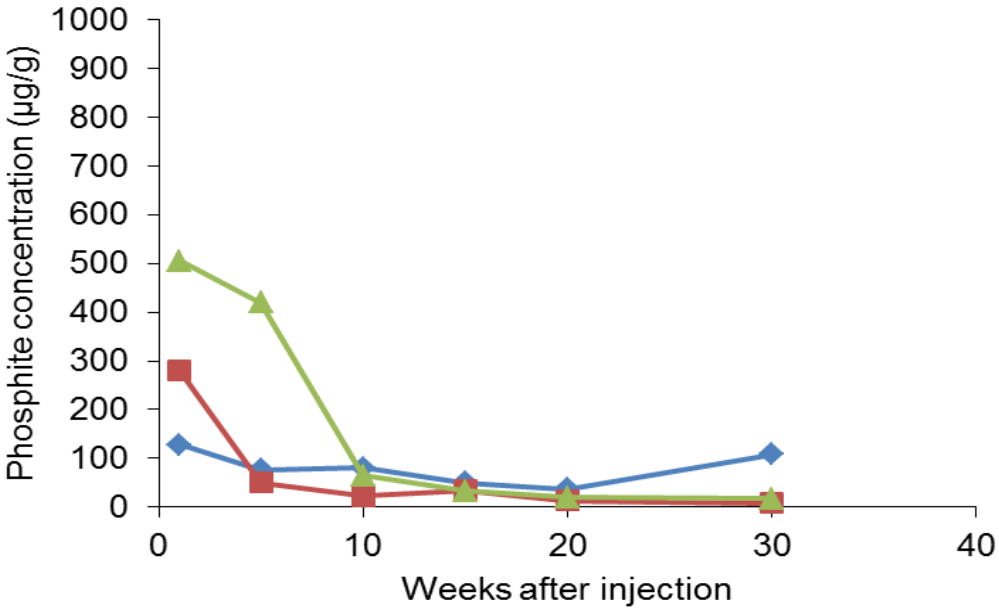

Figure 4-4. Phosphite concentration ($\mu g \cdot g^{-1}$) in old leaflets of coconut through 30 weeks following trunk injection with 3 rates of potassium phosphite: 30mL potassium phosphite in 90mL DI (◆), 60mL potassium phosphite in 180mL DI(■), and 90mL potassium phosphite in 270mL DI (▲).

Table 4-1.Phosphite contents (average of 5 replicates) in different parts of coconut palm at 40 weeks after injection with 90 mLpotassium phosphite in 270 mL DI.

Position	Mean ($\mu g \cdot g^{-1}$ ± SE)
Spear	249.45 ± 17.86
Young basal rachis	420.22 ± 23.05
Young distal rachis	234.98 ± 19.96
Young basal leaflets	146.04 ± 17.86
Young distal leaflet basal half	25.04 ± 19.96
Young distal leaflet distal half	13.11 ± 17.86
Old basal rachis	321.63 ± 23.05
Old distal rachis	64.73 ± 17.86
Old basal leaflets	18.49 ± 17.86
Old distal leaflets	16.10 ± 17.86

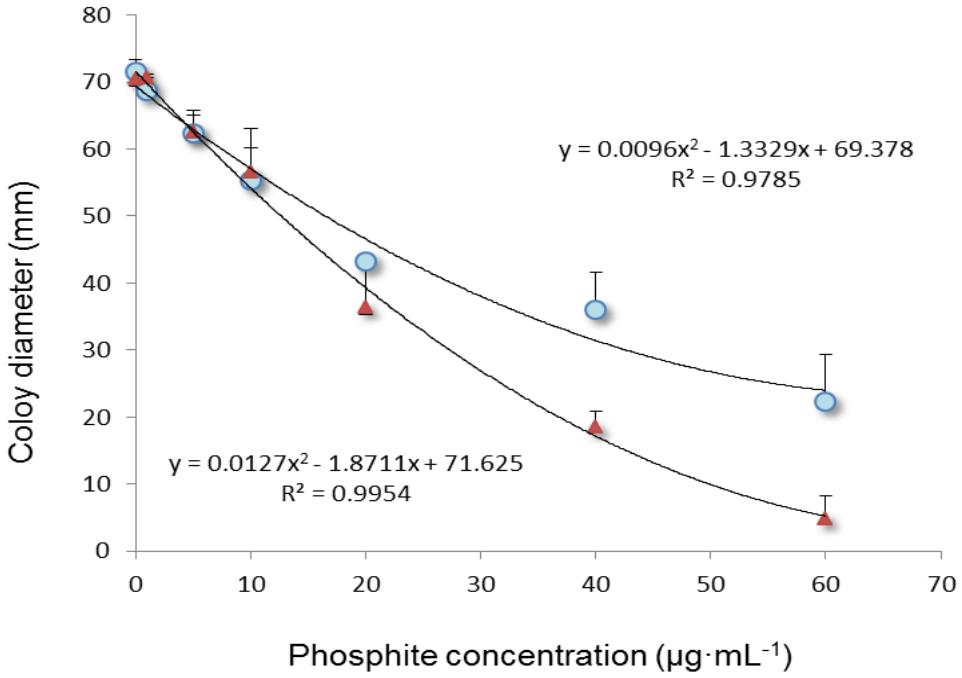

Figure 4-5. Colony diameters (mean of 8 replicates) of two isolates of *F. o. palmarum:* PLM-249A (●) and PLM-140B (▲) grown in1/5 PDA medium containing potassium phosphite.

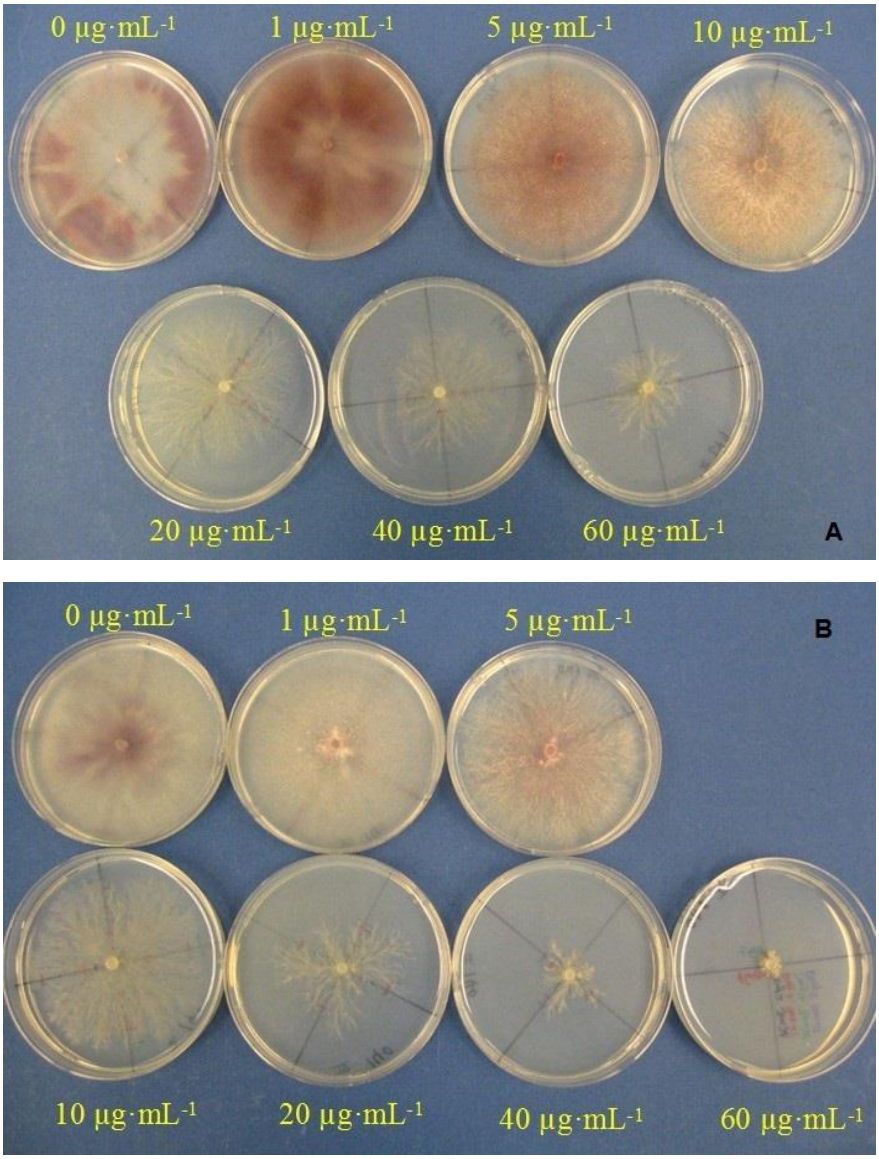

Figure 4-6. Growth of PLM-249A (A) and PLM- 140B (B) mycelium on 1/5 PDA with seven phosphite concentrations (0, 1, 5, 10, 20, 40, or 60 µg·mL^{-1}).

Table 4-2. Mortality (%) of *Washingtonia robusta* seedlings at 14 weeks after first treatment in trial 1.

Treatment	Application frequency	Mean of phosphite concentration in the first new leaf ($\mu g \cdot g^{-1}$)[z]	Mortality (%)
0.74 $\mu g \cdot mL^{-1}$ foliar spray	every 3 weeks	265.65	60
1.48 $\mu g \cdot mL^{-1}$ foliar spray	every 3 weeks	314.00	75
0.74 $\mu g \cdot mL^{-1}$ soil drench	every 3 weeks	209.60	25
1.48 $\mu g \cdot mL^{-1}$ soil drench	every 3 weeks	242.00	75
control		197.92	60

[z]Values are not significantly different based on mean separation using Tukey's HSD (α=0.05). There were 8 replicate samples per treatment.

Table 4-3. Mortality (%) of *Washingtonia robusta* seedlings at 14 weeks after first treatment in trial 2.

Treatment	Application frequency	Mean of phosphite concentration in the first new leaf ($\mu g \cdot g^{-1}$)[y]	Mortality (%)	Average mortality (%)[z]
0.74 $\mu g \cdot mL^{-1}$ foliar spray	every 3 weeks	377.70 a	12.5	6.25
1.48 $\mu g \cdot mL^{-1}$ foliar spray	every 3 weeks	330.49 a	0	
1.48 $\mu g \cdot mL^{-1}$ soil drench	every 3 weeks	131.63 bc	12.5	20.83
0.74 $\mu g \cdot mL^{-1}$ foliar spray	One time	146.96 bc	12.5	
1.48 $\mu g \cdot mL^{-1}$ foliar spray	One time	187.54 b	37.5	
0.74 $\mu g \cdot mL^{-1}$ soil drench	One time	69.36 c	37.5	31.25
1.48 $\mu g \cdot mL^{-1}$ soil drench	One time	78.62 c	62.5	
0.74 $\mu g \cdot mL^{-1}$ soil drench	every 3 weeks	77.27 c	12.5	
control		76.59 c	12.5	

[y]Values followed by the same letter are not significantly different based on mean separation using Tukey's HSD (α=0.05). There were 8 replicate samples per treatment.
[z]Average mortality is based on the 3 groups shown.

CHAPTER 5
CONCLUSION

Trunk injected potassium phosphite can be translocated by coconut palms immediately after application. Phosphite concentrations in old leaf rachis and all leaflet tissue reached their peaks within a week after application. However, phosphite concentrations in the spear leaf required 10 weeks to reach their highest concentrations. Spear leaves maintained a fairly high concentration for at least 10 months. Regardless of phosphite application rate, phosphite concentrations became stable ten weeks after application. Phosphite is not evenly distributed in coconut palms, but was concentrated in the spear leaf, young leaf rachis, old leaf rachis base, and young leaf basal leaflet tissues.

In vitro, PLM-249A and PLM-140B showed sensitivity to phosphite. As the concentration of phosphite was increased, growth of both isolates decreased. *In vivo, W. robusta* seedling mortality was reduced at an average foliar phosphite concentration of 350.49µg·g^{-1} when applied as a foliar spray every 3 weeks.

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BIOGRAPHICAL SKETCH

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