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Uptake, Movement, and Persistence of Fungicides in Mature Coconut Palms in Florida, U.S.

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Abstract. Palms are arborescent monocotyledons, with a vascular system different from eudicotyledonous trees. Compared to broadleaf trees, very little is known about the uptake, movement and persistence of systemic fungicides into the palm canopy. In this study, conducted in 2010 and 2012, four systemic fungicides were examined in coconut palms (*Cocos nucifera*) in Florida, U.S., using three different application methods. A bioassay method was used to detect the fungicides every four to five weeks in palm rachises located throughout the canopy. Thiophanate methyl, which can only be applied as a soil drench, was never detected. The same was true when propiconazole and thiabendazole were applied as soil drenches. Tebuconazole, applied via infusion, was also never detected, but this appeared to be due to formulation issues. Propiconazole was detected in only two of four palms in 2010, when applied via infusion. The labeled rate had increased by 2012, and when this new rate was applied via pressure injection, the fungicide was detected in all four replicate palms. Thiabendazole, when applied via infusion or pressure injection, was detected in all four replicate palms in both years. Propiconazole and thiabendazole persisted uniformly in the canopy for at least eight weeks after application, but amounts tapered off after that time. Neither fungicide was detected in any portion of the canopy after 28 weeks. Both fungicides were detected in leaves that emerged after their application. This suggests that these fungicides may be useful for controlling some canopy diseases.

Key Words. Coconut Palm; *Cocos nucifera*; Fungicide; Infusion; Palms; Pressure Injection; Propiconazole; Systemic Fungicides; Tebuconazole; Thiabendazole; Thiophanate Methyl.

Palms are an important landscape element throughout the state of Florida, in the United States. Unfortunately, they are increasingly affected by known pathogens and new or previously unrecognized fungal and fastidious bacterial pathogens (Downer et al. 2009; Elliott 2009). For two groups of lethal pathogens, phytoplasmas and *Phytophthora* spp., preventive disease management with trunk-injected chemicals is well-documented, and information about the chemical uptake, movement, and persistence has been determined (McCoy 1974; McCoy 1976; DeFranqueville and Renard 1989; Thévenin et al. 1995; Pohe et al. 2003; Yu et al. 2015). This is not the case with the true fungal pathogens, such as the *Fusarium* wilt pathogens, or the rachis and petiole blight pathogens.

The phytoplasma diseases of palms in the United States and Caribbean Basin, lethal yellowing and Texas Phoenix palm decline, are preventively managed by injecting the trunks of susceptible palms with the antibiotic oxytetracycline HCl. The amount, method, and timing was developed in the 1970s in response to a coconut palm (*Cocos nucifera*) lethal-yellowing epidemic in southern Florida and the Caribbean Basin (McCoy 1974; McCoy 1976). This research determined that a trunk injection was the most effective method for uptake of the antibiotic, which was moved throughout the palm canopy (leaf tissue), but antibiotic concentrations slowly declined to levels too low to be efficacious against the phytoplasma. Thus, the injection needed to be repeated every three to four months. Persistence in leaf tissue was determined via a biological assay.

Likewise, it has been determined that *Phytophthora* bud rot can be prevented by trunk injection of phosphite compounds (DeFranqueville and Renard 1989; Thévenin et al. 1995; Pohe et al. 2003), but uptake, movement, and persistence of phosphite in palms had not been examined until recently. Yu et al. (2015) determined that the likely reason phosphite injections are effective against *Phytophthora* bud rot is because the phosphite level

continued to increase over time in the youngest, unopened leaf, the initial target site of the pathogen, and persisted in this tissue for at least one year.

There have also been numerous studies examining the uptake, movement, and persistence of insecticides in palms (e.g., Kaakeh 2006; Ali and Caldwell 2010; Dembilio et al. 2014), but very few examining systemic fungicides. Fungicide studies have been conducted with some of the benzimidazole compounds, but with products that are no longer available in the United States (benomyl and carbendazim phosphate). Benomyl was used as a soil drench on adult Canary Island date palm (*Phoenix canariensis*), and residue was detected in the leaves via a bioassay (Surico 1977). Carbendazim phosphate was used as a trunk injection of adult Canary Island date palms, with the fungicide detected at 48–56 hours after the injection, which was the only time samples were obtained (Feather 1982). This fungicide was detected in the trunk and apical meristem, but not leaf tissue. A recent methods paper examined detection of multiple fungicides, including the benzimidazole fungicides carbendazim, thiabendazole, and thiophanate methyl after coconut trunk injection (Ferreira et al. 2015). However, samples were again obtained only once, at 45 hours after injection, and then only from the trunk. Carbendazim and thiabendazole were detected, but thiophanate methyl was not.

While fungicide uptake, movement, and persistence has been examined in eudicotyledonous trees, this information is difficult to extrapolate to palms, as palms are arborescent monocotyledons. Palm trunks are composed of thousands of vascular bundles, with each bundle containing xylem vessels, phloem sieve tube cells, and fibers, the numbers of which are essentially predetermined as palms have no vascular cambium (Tomlinson 1990; Tomlinson et al. 2011; Renninger et al. 2013). The directional orientation of vascular bundles is referred to as the "Rhapis principle," and means that bundles starting near the outside of the trunk near the soil line curve into the middle of the stem and then curve to the outside of the stem, and so forth up to the leaf canopy (Zimmerman and Tomlinson 1965). Furthermore, vascular bundles produce short branches that connect to other vascular bundles (Zimmerman 1973). This means that only one trunk injection site is needed to disperse pesticides uniformly throughout the palm canopy versus the multiple injection sites needed for eudicot trees.

Another feature of palms that may influence pesticide persistence is the fact that they are not deciduous, but are constantly shedding old leaves as new leaves emerge from the apical meristem. However, the anatomical structure of the palm ensures vascular connections are made with new leaves, because tyloses are formed to plug the protoxylem of abscising leaves (Zimmerman and Tomlinson 1965; Zimmerman 1973; Zimmerman and Tomlinson 1972). Thus, if products are taken up by the palm, the product may continue to move into new leaves, but will eventually become diluted as more new leaves emerge from the apical meristem and older leaves abscise. This would especially be expected with xylem-mobile pesticides.

The objectives of the study described herein were twofold: i) determine uptake, movement, and persistence of four systemic fungicides—propiconazole, tebuconazole, thiabendazole, and thiophanate methyl—in mature coconut palms; and ii) compare uptake, movement, and persistence of root drench versus trunk injection of propiconazole and thiabendazole in mature coconut palms. This information could then be used to plan landscape experiments with mature, tall palms affected by diseases such as *Fusarium* wilt, petiole blight, and rachis blight.

Thiophanate methyl was evaluated, as it is a standard product used as a root drench on palms in the landscape in Florida. It is not an injectable fungicide. For large palms (those with more than 1.5 to 2 m of clear trunk), foliar application of fungicides is impractical and spray drift a major hazard in the landscape. Injectable fungicides are desirable for palms as they limit exposure of the fungicide to humans and the environment. Propiconazole, tebuconazole, and thiabendazole were selected because they are currently registered for use as trunk injections in the U.S., although not necessarily for use in palms.

MATERIALS AND METHODS

Palms

The 18 coconut palms selected for the experiments were located at the University of Florida's Fort Lauderdale Research and Education Center (Davie, Florida, U.S.). The palms were not a

named cultivar, as they had been grown from non-certified seed. However, their characteristics were most closely associated with the Malayan Dwarf cultivar. These palms are growing in a uniform Margate fine sand soil and had not been subjected to any pesticide treatments prior to this experiment. Since 2005, the palms had been fertilized four times each year with 3.15 kg per palm per application of a controlled-released fertilizer (Lesco, Cleveland, Ohio, U.S.), 8N-0.9P-10K-4Mg plus micronutrients (2.0% Mn; 0.15% B, Fe, and Zn; 0.05% Cu). All palms had trunks at least 5.5 m high with at least 15 leaves. The palms were 4 m apart in all directions. Mean diameter at breast height (dbh) was 25 cm (range of 20 to 32 cm). Since dbh in palms does not correlate with canopy size or leaf number, and the goal was to determine if fungicides move into the leaves, it was decided to use the mean dbh (25 cm) for calculating fungicide application rates. Leaves used for sampling were numbered by starting with the newest growth at the time the fungicides were applied. The emerging spear leaf was designated leaf 0, the next-oldest leaf as leaf 1, and so on down through the canopy to the oldest leaf. Leaves that emerged after the start of the experiment were labeled with negative numbers (-1, -2, etc.) (see Figure 1).

The first experiment was initiated in August 2010. The second experiment used the same palms but was not initiated until July 2012 to allow for a completely new canopy of leaves to develop, which would also ensure there was no fungicide residual.

Fungicides and Application Methods

2010

Four fungicides were evaluated in 2010: 1) thiabendazole at 52.2 g a.i. per palm (Arbotect® 20-S; 220 g a.i. per liter; Syngenta Crop Protection, Inc., Greensboro, North Carolina, U.S.); 2) propiconazole at 15.6 g a.i. per palm (Alamo®, 156 g a.i. per liter; Syngenta Crop Protection, Inc., Greensboro, North Carolina, U.S.); 3) tebuconazole at 5 g a.i. per palm (Tebuject™ 16; 168 g a.i. per liter; Mauget, Inc., Arcadia, California, U.S.); 4) thiophanate methyl at 21.3 g a.i. per palm (3336®F; 479 g a.i. per liter; Cleary Chemical Corporation, Dayton, New Jersey, U.S.). There were four palms per fungicide treatment, and two palms for the untreated control treatment. Treatments were randomly assigned to the palms.

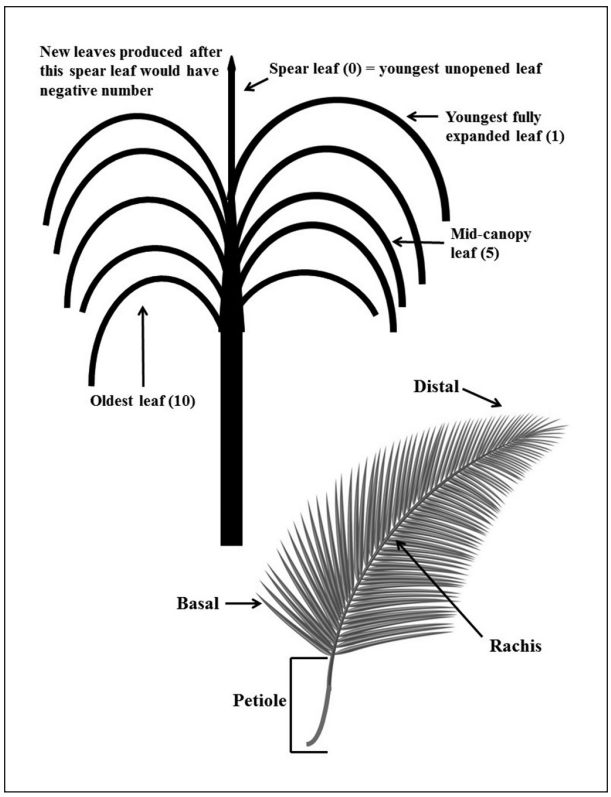

Figure 1. Location of palm tissues sampled for fungicide bioassay. Figure reprinted with permission of M.L. Elliott and *HortScience*.

To repeat and expand on the initial study conducted with thiabendazole (Elliott and Broschat 2012), thiabendazole, propiconazole, and tebuconazole fungicides were applied passively using pine tree infusers provided by Rainbow Treecare™ Scientific Advancements (Minneapolis, Minnesota, U.S.). The fungicides were not diluted prior to application. Two holes on opposite sides of the trunk at breast height were drilled 8.5 cm deep using a ~0.2 cm drill bit. The infuser nozzle was tapped into the trunk with a rubber mallet to a 2.5 cm depth, and the capped tube attached to the nozzle was tied upright to the trunk with flagging tape. Since each infuser only held 40 ml, the liquid was replenished in the infusers as the liquid was taken up by the palm. Infusers were removed after 30 hours.

Thiophanate methyl was applied as a root drench. To prevent turfgrass and weed roots from intercepting the fungicide, glyphosate was sprayed on a 3.3 m^2 area, with the palm in the center, two weeks prior to the drench. The fungicide was mixed with water, and each palm received 36 L of the fungicide mix applied with

a bucket to a 2.3 m^2 area, with the palm in the center. The ground was moist, as it had rained the night before the root-drench application.

2012

Based on knowledge gained in the 2010 experiment, only thiabendazole and propiconazole were evaluated in 2012, as thiophanate methyl was not detected in the leaf tissue in 2010 and the tebuconazole formulation was not compatible with infusion or injection into palms. The thiabendazole rate (52.2 g a.i. per palm) and formulation was the same as in 2010, but the propiconazole rate was doubled to 31.2 g a.i. per palm, using the same formulation. The fungicides were applied using two methods, and the applications were completed by noon.

For trunk uptake, instead of passive uptake of the fungicides using the pine tree infuser system, palms were injected with the Tree I.V. Micro Infusion® system (Arborjet, Inc., Woburn, Massachusetts, U.S.). One hole, located ~1 m from the soil line, was drilled 5 cm deep into the trunk using ~0.7 cm drill bit. A #3 Arborplug® was tapped into the hole, a bottle containing the fungicide was attached per instructions, and the system was pressurized to 207 kPa using the pump provided. All fungicides were taken up by the palms within four hours. A preliminary test demonstrated that the thiabendazole and propiconazole did not have to be diluted with water in order to be efficiently taken up by the palm via pressurized injection.

Each fungicide was also applied as a root drench. To prevent turfgrass and weed roots from intercepting the fungicide, glyphosate was sprayed on a 3.3 m^2 area, with the palm in the center, two weeks prior to the drench. The fungicide was mixed with water, and each palm received 36 L of the fungicide mix applied with a bucket to a 2.3 m^2 area, with the palm in the center. The ground was moist, as it had rained the night before the root-drench application.

There were four palms per fungicide x application treatment, and two palms for the untreated control treatment. Treatments were randomly assigned to the palms.

Bioassay for Fungicide Detection

A bioassay method was used to detect the fungicides in the leaf tissue sampled (Elliott and Broschat 2012). The fungus used was a *Penicillium* sp. (PLM-445), which is sensitive to all four fungicides. Spore suspensions were prepared in sterile deionized water, diluted to 10^4 spores per ml and added to sterile water agar. A thin layer of this agar-spore suspension was spread on the surface of 1/5 strength potato dextrose agar amended with 300 µg/ml streptomycin sulfate to inhibit bacterial growth. The fungal-seeded media was used immediately. After leaf tissue, obtained as described herein, or fungicide-saturated, paper discs were placed on the media, plates were incubated at 25°C in the dark. After 40 hours incubation, zones of fungal inhibition were measured in two directions and the average was recorded.

Standard inhibition curves were developed using sterile filter paper discs (6-mm diameter) saturated with a range of known concentrations of each fungicide. After drying, discs were placed on fungal-seeded media. Regression analysis was performed to obtain the equation that best fit the data for the standards at each sampling date. This equation was then used to calculate the amount of fungicide detected in the palm tissue.

For sampling the rachis tissue, two 10-cm segments were obtained from each leaf: i) basal portion of the rachis (BR), located about 45 cm from beginning of the rachis, and ii) distal portion of the rachis (DR), located about 45 cm from leaf tip (see Figure 1). The epidermis of each segment was removed and a cross section selected and cut into 5-mm by 5-mm pieces of ~2-mm thickness. There were four sections from each of the two segments placed on each of the fungal-seeded media in 2010, and six sections from each of the two segments in 2012. Note that sampling the rachis is destructive sampling, since the leaf is removed from the canopy. On each sample date, one of the oldest (=lower) leaves, a mid-canopy leaf (=middle), and the youngest, fully expanded leaf (=upper) was sampled from each palm.

The 2010 experiment was initiated on 16 August. Leaf samples were obtained on 13 Sep-

tember (leaves 10, 5, and 1), 11 October (leaves 9, 4, and 0), 8 November (leaves 8, 3, and -1), 6 December (leaves 7, 2, and -2), 1 March 2011 (leaves 6 and -3), and 12 April 2011 (leaf -4).

The 2012 experiment was initiated on 30 July. Leaf samples were obtained on 20 August (leaves 10, 5, and 1), 24 September (leaves 9, 4, and 0), 26 October (leaves 8, 3, and -1), 3 December (leaves 7, 2, and -2), and 11 February 2013 (leaves 6 and -3).

In both experiments, an aerial lift device was used to obtain the leaf samples. Leaf bases were numbered with a permanent black marker before the first samples were obtained. After the initial leaves were removed, their petiole stubs served as markers as well.

RESULTS

In both experiments, none of the control palms' tissue inhibited the *Penicillium* bioassay species in the first sampling (data not shown). Therefore, control palms were no longer sampled at subsequent dates in each experiment.

In 2010, tebuconazole and thiophanate methyl were not detected in any leaf in the canopy at any sampling date (data not shown). Propiconazole was detected in two of the four palms (Table 1). For Replicate Palm I, the fungicide was detected in the basal portion of the BR of all three leaves sampled at four and eight weeks after infusion. At 12 and 16 weeks, the product was only detected in the BR of the lower and upper leaves. Detection in the distal portion of the DR of this palm only occurred at four weeks (lower leaf only) and eight weeks (lower and upper leaves only). For Replicate Palm IV, the fungicide was detected in all three leaves in both portions of the leaf at 4, 8, and 12 weeks (Table 1). At 16 weeks, propiconazole was only detected in the BR and DR of the middle leaf. Propiconazole was not detected in any leaf at 28 and 34 weeks after infusion (data not shown).

Thiabendazole was detected in all four replicate palms in 2010 on all four sampling dates, although the amount detected varied widely among palms, with the greatest amount routinely detected in Replicate Palm III (Table 2). The only tissue in which thiabendazole was not detected across all four replicate palms was the DR of all three leaves at 16 weeks. As with propiconazole, thiabendazole was not detected in any leaf at 28 and 34 weeks after infusion (data not shown).

Table 1. Amount (µg/g palm tissue) of propiconazole (Alamo) detected in *Cocos nucifera* leaf rachis tissue at 4, 8, 12, and 16 weeks after application via passive infusion in 2010.

		Mean[z] ± SD (µg/g)							
		4 weeks		8 weeks		12 weeks		16 weeks	
Palm replicate	Leaf location in canopy[y]	Basal rachis	Distal rachis	Basal rachis	Distal rachis	Basal rachis	Distal rachis	Basal rachis	Distal rachis
I	Lower	47 ± 5	29 ± 19	47 ± 15	43 ± 5	42 ± 7	0 ± 0	8 ± 6	0 ± 0
	Middle	12 ± 25	0 ± 0	49 ± 10	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Upper	55 ± 6	0 ± 0	50 ± 6	51 ± 5	45 ± 8	0 ± 0	30 ± 2	0 ± 0
II	Lower	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Middle	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Upper	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
III	Lower	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Middle	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Upper	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
IV	Lower	87 ± 29	49 ± 0	71 ± 5	51 ± 5	77 ± 15	42 ± 4	0 ± 0	0 ± 0
	Middle	86 ± 33	7 ± 14	88 ± 6	50 ± 2	55 ± 9	44 ± 7	37 ± 4	41 ± 0
	Upper	74 ± 2	80 ± 7	78 ± 8	86 ± 7	45 ± 8	38 ± 3	0 ± 0	0 ± 0
				Mean[x] ± SE					
	Lower	34 ± 38	20 ± 22	30 ± 31	23 ± 24	30 ± 33	10 ± 18	2 ± 8	0 ± 0
	Middle	25 ± 40	2 ± 7	34 ± 37	14 ± 24	14 ± 24	11 ± 19	9 ± 16	10 ± 18
	Upper	32 ± 33	20 ± 35	32 ± 34	34 ± 37	22 ± 23	10 ± 17	8 ± 13	0 ± 0

[z] Each value is the mean of four replicate cross sections of leaf rachises.

[y] Lower leaves were leaf numbers 10, 9, 8, and 7; middle leaves were leaf numbers 5, 4, 3, and 2; upper leaves were leaf numbers 1, 0, -1, and -2, at 4, 8, 12, and 16 weeks, respectively.

[x] Mean of 16 replicate cross sections of leaf rachises, across all four replicate palms.

Table 2. Amount (µg/g palm tissue) of thiabendazole (Arbotect) detected in *Cocos nucifera* leaf rachis tissue at 4, 8, 12, and 16 weeks after application via passive infusion in 2010.

		Mean ± SD[z] (µg/g)							
		4 weeks		8 weeks		12 weeks		16 weeks	
Palm replicate	Leaf location in canopy[y]	Basal rachis	Distal rachis	Basal rachis	Distal rachis	Basal rachis	Distal rachis	Basal rachis	Distal rachis
I	Lower	53 ± 4	0 ± 0	95 ± 11	23 ± 2	65 ± 27	0 ± 0	0 ± 0	0 ± 0
	Middle	35 ± 16	0 ± 0	47 ± 17	0 ± 0	48 ± 11	0 ± 0	34 ± 3	0 ± 0
	Upper	81 ± 3	44 ± 8	79 ± 19	96 ± 6	59 ± 12	66 ± 5	69 ± 13	0 ± 0
II	Lower	27 ± 18	27 ± 5	27 ± 19	0 ± 0	34 ± 28	0 ± 0	25 ± 17	0 ± 0
	Middle	84 ± 9	0 ± 0	124 ± 11	0 ± 0	92 ± 12	0 ± 0	51 ± 13	0 ± 0
	Upper	94 ± 4	17 ± 20	153 ± 18	58 ± 16	118 ± 21	42 ± 16	89 ± 11	0 ± 0
III	Lower	120 ± 20	97 ± 13	139 ± 17	92 ± 4	149 ± 26	56 ± 5	73 ± 17	0 ± 0
	Middle	105 ± 14	73 ± 9	148 ± 4	96 ± 14	153 ± 60	70 ± 17	79 ± 121	0 ± 0
	Upper	123 ± 6	102 ± 12	125 ± 24	147 ± 25	222 ± 18	106 ± 13	108 ± 33	0 ± 0
IV	Lower	32 ± 10	10 ± 20	46 ± 4	0 ± 0	17 ± 19	0 ± 0	0 ± 0	0 ± 0
	Middle	40 ± 2	22 ± 14	101 ± 17	50 ± 24	71 ± 12	0 ± 0	23 ± 18	0 ± 0
	Upper	52 ± 19	53 ± 11	82 ± 18	80 ± 9	102 ± 10	38 ± 6	61 ± 14	0 ± 0
				Mean[x] ± SE					
	Lower	58 ± 39	34 ± 40	77 ± 46	29 ± 38	66 ± 56	14 ± 24	25 ± 32	0 ± 0
	Middle	66 ± 31	24 ± 31	105 ± 39	37 ± 42	91 ± 47	18 ± 31	60 ± 44	0 ± 0
	Upper	88 ± 27	54 ± 33	110 ± 35	95 ± 35	125 ± 62	63 ± 29	82 ± 25	0 ± 0

[z] Each value is the mean of four replicate cross sections of leaf rachises.
[y] Lower leaves were leaf numbers 10, 9, 8, and 7; middle leaves were leaf numbers 5, 4, 3, and 2; upper leaves were leaf numbers 1, 0, -1, and -2, at 4, 8, 12, and 16 weeks, respectively.
[x] Mean of 16 replicate cross sections of leaf rachises, across all four replicate palms.

In 2012, the rate of propiconazole was doubled, and both propiconazole and thiabendazole were injected under pressure into the palm trunk or were applied at equivalent rates as root drenches. When these fungicides were applied as root drenches, they were not detected at any sampling date in any leaf tissue (data not shown).

For propiconazole, the fungicide was detected in both the BR and DR in all three leaves and all four replicate palms at three and eight weeks after injection (Table 3). At 13 weeks, the fungicide was detected in all leaves and leaf tissue except for the DR of the youngest (upper) leaf of one palm. At 18 weeks, the fungicide was detected in the BR of the middle and lower leaves of all four palms, but only in the DR for middle and lower leaves of two replicate palms, and not in any of the youngest leaves (BR or DR). Propiconazole was not detected in any leaf tissue 28 weeks after injection (data not shown).

Thiabendazole was detected in all tissue samples of the four replicate palms in 2012 at three and eight weeks after injection (Table 4). At 13 weeks, thiabendazole was detected in all leaf tissue of Replicate Palm II, but it was not detected in any leaves of Replicate Palm I, only in the BR of the upper leaf of Replicate Palm III, and only in the BR of the middle and upper leaves of Replicate Palm IV. At 18 weeks, thiabendazole was only detected in the BR and DR of the lowest leaf of Replicate Palm II. Thiabendazole was not detected in any leaf tissue 28 weeks after injection (data not shown).

No evidence of phytotoxicity was observed due to any fungicide treatment at any time in the two experiments. Neither was there evidence of external trunk damage beyond the initial holes drilled for infusion or injection.

DISCUSSION

Systemic fungicides applied by infusion or injection to control diseases of hardwood trees are not new. They were first evaluated for control of Dutch elm disease (Haugen and Stennes 1999; Stennes 2000) and later used for management of oak wilt (Koch et al. 2010). Other tree diseases for which this technology has been studied include ash dieback (Dal Maso et al. 2014), *Armillaria* root rot (Adaskaveg et al. 1999), and madrone canker (Elliott and Edmonds (2008).

The fungicides used in this experiment belong to two major chemical groups. Thiophanate methyl and thiabendazole are methyl benzimidazole car-

Table 3. Amount (µg/g palm tissue) of propiconazole (Alamo) detected in *Cocos nucifera* leaf rachis tissue at 3, 8, 13, and 18 weeks after application via pressurized injection in 2012.

		Mean[z] ± SD							
		3 weeks		8 weeks		13 weeks		18 weeks	
Palm replicate	Leaf location in canopy[y]	Basal rachis	Distal rachis	Basal rachis	Distal rachis	Basal rachis	Distal rachis	Basal rachis	Distal rachis
I	Lower	87 ± 12	56 ± 3	57 ± 9	62 ± 5	41 ± 4	31 ± 3	Detected	0 ± 0
	Middle	72 ± 13	44 ± 7	56 ± 6	53 ± 4	43 ± 5	39 ± 2	Detected	0 ± 0
	Upper	69 ± 7	56 ± 9	54 ± 11	49 ± 4	33 ± 4	0 ± 0	0 ± 0	0 ± 0
II	Lower	105 ± 20	100 ± 14	97 ± 11	107 ± 9	76 ± 7	94 ± 7	57 ± 8	67 ± 5
	Middle	110 ± 13	111 ± 6	102 ± 5	111 ± 5	76 ± 5	69 ± 7	59 ± 15	54 ± 5
	Upper	105 ± 10	108 ± 9	76 ± 12	72 ± 10	54 ± 12	44 ± 7	0 ± 0	0 ± 0
III	Lower	101 ± 6	75 ± 8	73 ±12	63 ± 5	50 ± 6	48 ± 5	49 ± 3	0 ± 0
	Middle	97 ± 10	85 ± 7	62 ± 5	77 ± 12	57 ± 6	39 ± 2	61 ± 20	0 ± 0
	Upper	70 ± 7	61 ± 5	60 ± 3	48 ± 6	48 ± 3	31 ± 5	0 ± 0	0 ± 0
IV	Lower	123 ±13	87 ± 9	94 ± 11	105 ± 12	74 ± 8	68 ± 11	62 ± 5	61 ± 11
	Middle	116 ± 20	107 ± 12	89 ± 12	112 ± 13	55 ± 13	71 ± 9	58 ± 3	68 ± 4
	Upper	115 ± 16	100 ± 11	85 ± 13	69 ± 7	58 ± 7	52 ± 7	0 ± 0	0 ± 0
				Mean[x] ± SE					
	Lower	104 ± 18	80 ± 19	80 ± 19	84 ± 23	61 ± 16	60 ± 24	56 ± 8	46 ± 34
	Middle	99 ± 22	86 ± 27	75 ± 19	85 ± 23	58 ± 14	54 ± 16	59 ± 13	31 ± 31
	Upper	90 ± 23	81 ± 24	69 ± 16	61 ± 14	48 ± 11	32 ± 21	0 ± 0	0 ± 0

[z] Each value is the mean of six replicate cross sections of leaf rachises. At 18 weeks, propiconazole was detected in some sections, but at <1 µg/g; therefore, it was not included in calculating the mean.

[y] Lower leaves were leaf numbers 10, 9, 8, and 7; middle leaves were leaf numbers 5, 4, 3, and 2; upper leaves were leaf numbers 1, 0, -1, and -2, at 3, 8, 13, and 18 weeks, respectively.

[x] Mean of 24 replicate cross sections of leaf rachises, across all four replicate palms, except at 18 weeks where the number of replicate cross sections would have been 18 for basal rachis tissue for all three leaf locations.

Table 4. Amount (µg/g palm tissue) of thiabendazole (Arbotect) detected in *Cocos nucifera* leaf rachis tissue at 3, 8, 13, and 18 weeks after application via pressurized injection in 2012.

		Mean[z] ± SD							
		3 weeks		8 weeks		13 weeks		18 weeks	
Palm replicate	Leaf location in canopy[y]	Basal rachis	Distal rachis	Basal rachis	Distal rachis	Basal rachis	Distal rachis	Basal rachis	Distal rachis
I	Lower	106 ± 12	109 ± 5	23 ± 3	25 ± 4	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Middle	111 ± 14	113 ± 8	31 ± 8	31 ± 7	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Upper	147 ± 14	141 ± 5	23 ± 4	25 ± 5	0 ± 0	0 ± 0	0 ± 0	0 ± 0
II	Lower	164 ± 17.3	165 ± 9	147 ± 11	118 ± 6	139 ± 11	109 ± 12	86 ± 16	39 ± 6
	Middle	177 ± 21	156 ± 12	127 ± 12	119 ± 8	109 ± 38	112 ± 7	0 ± 0	0 ± 0
	Upper	155 ± 16	95 ± 9	130 ± 13	73 ± 7	27 ± 7	46 ± 4	0 ± 0	0 ± 0
III	Lower	116 ± 5	138 ± 13	28 ± 9	30 ± 3	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Middle	103 ± 4	129 ± 7	30 ± 9	23 ± 3	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Upper	117 ± 7	118 ± 11	28 ± 10	20 ± 2	15 ± 9	0 ± 0	0 ± 0	0 ± 0
IV	Lower	112 ± 19	93 ± 9	76 ± 22	64 ± 5	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Middle	125 ± 17	102 ± 17	81 ± 7	66 ± 12	23 ± 16	0 ± 0	0 ± 0	0 ± 0
	Upper	110 ± 15	47 ± 6	51 ± 15	56 ± 11	17 ± 9	0 ± 0	0 ± 0	0 ± 0
					Mean[x] ± SE				
	Lower	125 ± 27	127 ± 28	68 ± 51	60 ± 37	35 ± 60	27 ± 48	21 ± 38	10 ± 17
	Middle	129 ± 32	124 ± 24	68 ± 41	62 ± 39	33 ± 48	28 ± 49	0 ± 0	0 ± 0
	Upper	132 ± 23	102 ± 34	58 ± 44	52 ± 22	15 ± 12	12 ± 20	0 ± 0	0 ± 0

[z] Each value is the mean of six replicate cross sections of leaf rachises.

[y] Lower leaves were leaf numbers 10, 9, 8, and 7; middle leaves were leaf numbers 5, 4, 3, and 2; upper leaves were leaf numbers 1, 0, -1, and -2, at 3, 8, 13, and 18 weeks, respectively.

[x] Mean of 24 replicate cross sections of leaf rachises, across all four replicate palms.

bamates, with thiophanate methyl in the thiophanates chemical group and thiabendazole in the benzimidazole chemical group (Delp 1995; FRAC 2016). Propiconazole and tebuconazole are demethylation inhibitor fungicides and are in the triazole chemical group (Kuck et al. 1995; FRAC 2016). All fungicides used in the current study were xylem-mobile systemic products. In theory, such products, when taken up by the root system, will move throughout the plant via the xylem.

All four fungicides are formulated differently. Arbotect 20-S is a soluble liquid concentrate of thiabendazole hypophosphate; Alamo is a microencapsulated emulsifiable concentrate of propiconazole; Tebuject 16 is an emulsifiable concentrate of tebuconazole; 3336F is a flowable concentrate of thiophanate methyl. While the first three products have been formulated for trunk and root-flare injection of trees, thiophanate methyl has not, probably due to the fact it does not form a solution in water. It was included in the study because it is a standard fungicide used on palms in the Florida landscape, not for root disease control but for canopy disease control, even though there are no studies regarding its effectiveness for disease control in mature palms.

While there was considerable variability in *Penicillium* inhibition by individual rachis pieces, the overall inhibition was relatively uniform in the leaf canopy for the first three sampling dates for both thiabendazole and propiconazole in both years. The *Penicillium* inhibition assay provided a uniform method of detecting the fungicides. In other words, different chemical analytical assays were not required for the detection of each fungicide. The QuEChERS method for homogenizing samples prior to analysis with ultra-high-performance liquid chromatography-tandem mass spectrometry may solve this problem (Ferreira et al. 2015). However, this method is time-consuming, requires dry ice for crushing, and expensive equipment and consumables.

In this study, thiophanate methyl, propiconazole, and thiabendazole were not detected in the palm canopy when applied as root drenches. The inability to detect thiophanate methyl in the canopy was not surprising. A study conducted on coconut in Brazil injected technical material of both thiabendazole and thiophanate methyl. After 45 hours, thiabendazole was detected but not thiophanate methyl (Ferreira et al. 2015). While the authors attributed this to the fact that thiophanate methyl is metabolized to carbendazim (as is benomyl), they did not consider the fact that thiophanate methyl is more stable than benomyl and would not have been broken down to carbendazim in less than 48 hours (Delp 1995). Plus, the level of carbendazim detected was not doubled, as would be expected if thiophanate methyl was being quickly metabolized to carbendazim as postulated.

The effectiveness of any of the benzimidazoles as a soil application has been poor, as the compounds are tightly adsorbed to soil colloids and organic matter (Delp 1995). Practical disease control has been obtained by application to potting substrates for container-grown plants, incorporation into plant beds, or in-furrow applications (Delp 1995). Mobility in soil is expected to be slight to none for propiconazole, slight for thiabendazole, and moderate for thiophanate methyl (NCBI 2015; NCBI 2016a; NCBI 2016b). This slight-to-no mobility in soil may explain why propiconazole and thiabendazole are not labeled for soil applications.

In the 2010 study, tebuconazole was not detected in the palm canopy. This was likely due to this formulation's incompatibility with compounds in the vascular bundles. Very little of the material was passively taken up by the palms due to the clogging of the nozzles. Attempts to inject the tebuconazole with pressure were also unsuccessful. Although this product has been used in hardwood trees, it may be necessary to develop a formulation that is more water compatible for injection into palms, which contain considerably more water than hardwood trees due to their vascular structure.

While thiabendazole and propiconazole were not detected in the palm canopy when applied as root drenches, they were detected in the palm canopy when passively infused or injected under pressure. Propiconazole was only detected in two of four replicate palms in the 2010 study, but was detected in all four replicate palms in the 2012 study. Although the method of application changed from 2010 (infusion) to 2012 (pressurized injection), it is more likely that the increased detection in 2012 was due to the increased amount of propiconazole used. This is presumed because the method of application did not affect thiabendazole detection, where the same amount of chemical was used in both years.

Between the 2010 and 2012 experiments, the label for Alamo (propiconazole) changed. The amount of material that could be used for a single dose was doubled, probably to increase the persistence of propiconazole in red bay (*Persea borbonia*) and avocado (*Persea americana*) (Mayfield et al. 2008; Ploetz et al. 2011). Research regarding laurel wilt disease, caused by *Raffaelea lauricola*, affecting members of the Lauraceae in southeastern U.S., had suggested that the persistence of propiconazole, when using the 2010 labeled rate, began to decline 4.5 months after root-flare injections (Mayfield et al. 2008).

For both propiconazole and thiabendazole, evidence of movement into new growth is demonstrated by the detection of the fungicides in the rachises of leaves labeled with a negative number; in other words, leaves that had not yet emerged at the time of the fungicide application. For example, in 2012, propiconazole was detected in the basal and distal rachis of leaf -1 of all four replicate palms. This leaf number was sampled 13 weeks after the fungicide was injected. Similar results were obtained for thiabendazole in both 2010 and 2012.

However, what is also evident is the decrease in propiconazole and thiabendazole in leaves in the palm canopy over time. With thiabendazole, this was evident in the distal rachis tissue at the 16-week post-application sampling in 2010, and even earlier in 2012 for three of the four replicate palms. For propiconazole in 2012, no or minimal material was detected in most leaf samples at 18 weeks. In both years, neither propiconazole or thiabendazole was detected in any leaf by 28 weeks after treatment (data not shown).

Research results with thiabendazole, including the current study as well as a previous study (Elliott and Broschat 2010), are fairly consistent with results obtained for thiabendazole used in elm trees, as summarized by Stennes (2000). First, a bioassay technique for detection of thiabendazole in plant tissue is feasible when using twig (hardwood) or rachis (palm) tissue. Second, the fungicide was evenly distributed in the canopy. Third, the fungicide could be detected in tissue not yet developed (hardwood) or fully developed (palm) at the time of the injection. The primary difference is the persistence of thiabendazole, which appears to be as long as 12 months in elm trees but less than 5 months for coconut palms.

Propiconazole persistence in coconut palms was also less than five months. Results with persistence of propiconazole in trunk vascular tissue of hardwood trees have been mixed. One study with red oak (*Quercus rubra*) demonstrated propiconazole was detected in trunk tissue 12 months after injection (Osterbauer and French 1992), but another study with red oak reported in Blaedow et al. (2010) indicated it was not. In general, fungicide persistence seems longer in hardwood trees than in palms (Stennes and French 1987; Osterbauer and French 1992; Mayfield et al. 2008; Blaedow et al. 2010). However, the fungicide persistence results in palms are not greatly different from the results obtained with oxytetracycline HCl injections (McCoy 1976).

Methods for consistently inoculating palms with pathogens and observing disease development in mature, tall palms in field nurseries have not been successful. Therefore, it is necessary to conduct research in large landscapes where diseases, such as *Fusarium* wilt, petiole blight, and rachis blight are naturally occurring. The results from this research will help predict which fungicides are likely to be effective for palm disease management.

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Résumé. Les palmiers sont des plantes monocotylédones arborescentes, avec un système vasculaire distinct de celui des plantes dicotylédones arborescentes. Comparativement aux arbres feuillus, on en connaît très peu sur l'absorption, le mouvement et la rémanence des fongicides systémiques appliqués à une canopée constituée de palmiers. Dans cette étude, menée en 2010 et en 2012, quatre fongicides systémiques ont été évalués sur des cocotiers (*Cocos nucifera*) en Floride, États-Unis, en utilisant trois différentes méthodes d'application. Une méthode d'essai biologique a été utilisée pour détecter la présence de fongicides, toutes les quatre à cinq semaines, sur les pétioles des palmes récoltés dans toute la canopée. Le thiophanate-méthyle, qui ne peut être appliqué que par trempage du sol, n'a jamais été détecté. Il en fut de même lorsque le propiconazole et le thiabendazole furent appliqués par trempage du sol. Le tebuconazole, appliqué par perfusion, n'a également jamais été détecté, mais cela semble être plutôt dû à des problèmes de formulation. Le propiconazole ne fut détecté que chez seulement deux des quatre palmiers en 2010 lorsqu'il avait été appliqué par perfusion. La dose prescrite sur l'étiquette a été augmentée en 2012, et lorsque cette nouvelle dose a été appliquée par injection pressurisée, la présence du fongicide fut détectée chez ces quatre mêmes palmiers. Le thiabendazole, appliqué tant par perfusion que par injection pressurisée, a été détecté chez ces quatre mêmes palmiers à chacune des deux années. Le propiconazole et le thiabendazole ont persisté uniformément dans la canopée pendant une durée d'au moins huit semaines suivant l'application, mais les quantités ont diminué après cette période. Aucun de ces deux fongicides n'a été détecté à aucun endroit de la canopée après 28 semaines. Ces deux fongicides furent détectés dans les feuilles qui émergèrent après leur application. Ce qui laisse supposer que ces fongicides peuvent être utiles pour contrôler certaines maladies de feuillage.

Zusammenfassung. Palmen sind verholzte Einkeimblättrige, deren vaskuläres System sich von den Bedecktsamern, bzw. der zweikeimblättrigen Pflanzen unterscheidet. Verglichen mit den Laubbäumen ist hier noch sehr wenig bekannt über die Aufnahme, Bewegung und Persistenz von systemischen Fungiziden in die Krone der Palmen. In dieser Studie, die 2010 und 2012 durchgeführt wurde, wurden vier systemische Fungizide in Kokospalmen in Florida, USA, unter Anwendung von drei verschieden Applikationsmethoden untersucht. Um die Fungizide alle vier bis fünf Wochen in den Palmwedeln aus verschiedenen Bereichen der Krone zu lokalisieren wurde eine biologische Testreihenmethode verwendet. Thiophanatmethyl, welches nur als Bodenkontaktmittel appliziert werden kann, wurde niemals nachgewiesen. Das erwies sich auch als zutreffend, wenn Propiconazol und Thiabendazol nur als Bodenkontaktmittel appliziert wurden. Tebuconazol, appliziert via Infusion, wurde auch nie nachgewiesen, aber das schien an der Formel zu liegen. Propiconazol wurde in 2010 nur in zwei von vier Palmen nachgewiesen, wenn es als Infusion appliziert wurde. Die markierte Rate stieg in 2012, und wenn diese neue Rate mit einer Druckinjektion appliziert wurde, erschien das Fungizid in allen vier Palmen in beiden Jahren. Propiconazol und Thiabendazol hielten sich gleichmäßig in der Krone für mindestens acht Wochen nach der Applikation, aber danach ließen die Mengen nach. Nach 28 Wochen wurde keines der Fungizide in irgendeinem Teil der Kronen nachgewiesen. Beide Fungizide wurden in den Blättern, die sich nach der Applikation öffneten, nachgewiesen. Das bedeutet, dass diese Fungizide bei der Kontrolle einiger Kronenkrankheiten nützlich sein können.

Resumen. Las palmas son monocotiledóneas arborescentes, con un sistema vascular diferente de los árboles dicotiledóneos. En comparación con los árboles de hoja ancha, se sabe muy poco sobre la captación, el movimiento y la persistencia de los fungicidas sistémicos en el dosel de la palma. En este estudio, realizado en 2010 y 2012, se examinaron cuatro fungicidas sistémicos en las palmas de coco (*Cocos nucifera*) en Florida, EE.UU., utilizando tres métodos de aplicación diferentes. Se utilizó un método de bioensayo para detectar los fungicidas cada cuatro a cinco semanas en los raquis de las palmas localizadas a lo largo de la copa. El tiofanato de metilo, que sólo se puede aplicar con zanjas en el suelo, nunca fue detectado. Lo mismo fue cierto cuando propiconazol y tiabendazol se aplicaron en el suelo. El tebuconazol, aplicado vía infusión, tampoco fue detectado, pero esto se debió a problemas de formulación. El propiconazol se detectó en sólo dos de cuatro palmas en 2010, cuando se aplicó mediante infusión. La tasa etiquetada fue aumentada en 2012, y cuando esta nueva tasa se aplicó a través de inyección a presión, el fungicida se detectó en las repeticiones de las cuatro palmas. El tiabendazol, cuando se aplicó vía infusión o inyección a presión, se detectó en las cuatro palmas replicadas en ambos años. El propiconazol y el tiabendazol persistieron uniformemente en el dosel durante al menos ocho semanas después de la aplicación, pero las cantidades disminuyeron después de ese tiempo. No se detectó fungicida en ninguna porción del dosel después de 28 semanas. Ambos fungicidas fueron detectados en las hojas que emergieron después de su aplicación. Esto sugiere que estos fungicidas pueden ser útiles para controlar algunas enfermedades de la copa.