Reevaluation of the Application Method and Efficacy of Propiconazole in Controlling Laurel Wilt in Avocado Orchards in South Florida

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Abstract. Propiconazole has fungistatic and fungicidal properties against *Harringtonia lauricola*, the causal agent of laurel wilt disease. Propiconazole injections are used by Florida, USA, farmers as a prophylactic method to manage the disease in avocado (*Persea americana*) trees, but its efficacy has remained questionable for more than a decade due to documented restricted mobility within the tree vascular system. This study was conducted to evaluate the absorption of propiconazole when using soil drenching as an alternative application method, assess the efficacy of propiconazole in controlling disease development when drenched or injected, and its synergistic effect on common cultural management practices used by the local farmers, such as branch removal and trunk cutting ("stumping"). To determine if propiconazole soil-drenching can provide better xylem coverage, potted and mature orchard trees were treated with different concentrations and artificially inoculated with the pathogen. Propiconazole translocation from the roots to above-the-ground tissue was confirmed in potted and orchard trees, but the concentrations in orchard trees were below the fungicidal threshold (1 ppm). Although none of the potted trees developed laurel wilt symptoms, all inoculated branches of the orchard trees did. Furthermore, noninoculated branches in more than 80% of the inoculated and propiconazole-treated orchard trees developed symptoms, even though the inoculated branch was cut at the early stages of disease development. To elucidate if propiconazole injections effectively control the disease, trees from a commercial orchard that were injected five times were challenged by artificial inoculation. Propiconazole concentration in trees was highly variable (ranging from < 0.01 to 294 ppm), but even in trees with a high concentration of propiconazole, inoculated and noninoculated branches developed symptoms. Even though drenched and injected trees were "stumped" soon after symptoms appeared in the noninoculated branches (4 to 5 months after inoculation), all of the stumps in the drenched plot and 80% of the injected trees, showed internal symptoms 5 and 4 months after the cut, respectively. Results demonstrate that the soil-drenching of propiconazole is an ineffective application method in orchard trees, and that the conventional injection does not prevent disease development after artificial inoculation. Moreover, because propiconazole does not prevent the movement of the pathogen to the trunk, the "stumping" of infected trees to reduce the disease in the orchard is an inadequate practice. This study highlights the critical need for other active ingredients with lower fungicidal thresholds, longer half-life, and higher xylem mobility.

Laurel wilt is a lethal vascular disease caused by *Harringtonia lauricola* (formerly *Raffaelea lauricola*), a fungal nutritional symbiont of the redbay ambrosia beetle (*Xyleborus glabratus*) and related species (Carrillo et al. 2014; Fraedrich et al. 2008; Harrington et al. 2008). This invasive pathogen, reported in the United States since 2004, affects several native and introduced species in the family Lauraceae and has caused significant damage to southeastern forest ecosystems (Fraedrich et al. 2008; Hughes et al. 2017; Ploetz et al. 2017a). Since 2012, when laurel wilt was first reported in a commercial avocado (*Persea americana*) orchard in the Miami-Dade area of Florida, USA, the disease has caused significant economic losses, killing at least 300,000 fruit-bearing trees (Evans et al. 2015; Crane JH, unpublished). To date, laurel wilt is present in all 67 Florida counties (Crane et al. 2020) and has been reported in 12 US states (Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, Texas, Virginia) (Southern Regional Extension Forestry, Forest Health 2023). The destructive nature of this disease, in combination with its multiple hosts and potential vectors, makes it an imminent threat to avocado production worldwide.

For the past 17 years, researchers at the Tropical Research and Education Center of the University of Florida [TREC (Homestead, FL, USA)] have investigated possible strategies to manage laurel wilt; unfortunately, only a few cultural practices have shown to be useful in reducing the vector population and disease pressure (Crane et al. 2016, 2020; Menocal et al. 2022). A common strategy to treat vascular diseases in trees is the use of systemic fungicides. Propiconazole is a systemic triazole fungicide used as a preventive treatment for vascular diseases such as oak wilt [*Bretziella fagacearum* (formerly *Ceratocystis fagacearum*)] and Dutch elm disease (*Ophiostoma novo-ulmi*) (Appel 1990; Appel and Kurdyla 1992; Osterbauer and French 1992; Stennes 2000). Through in vitro assays, Mayfield et al. (2008) showed that propiconazole (Alamo®; Syngenta, Greensboro, NC, USA) inhibited the growth of *H. lauricola* at concentrations of 0.01 (84% inhibition) and 0.1 ppm (100% inhibition), and was fungitoxic at 1 ppm. The authors also reported that redbay trees (*Persea borbonia*) treated with propiconazole, using the maximum label rate (1.2 g a.i. per inch trunk diameter) and delivered as macro-infusion (fungicide

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29,574	fl oz	μL	3.3814×10^{-5}
29.5735	fl oz	mL	0.0338
0.3048	ft	m	3.2808
0.0929	ft^2	m^2	10.7639
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
0.4536	lb	kg	2.2046
1.1209	lb/acre	$kg \cdot ha^{-1}$	0.8922
28.3495	oz	g	0.0353
1	ppm	$mg \cdot kg^{-1}$	1
(°F − 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

diluted and injected into root flares), remained asymptomatic for at least 30 weeks after trees were inoculated with the pathogen.

The effectiveness of propiconazole for managing laurel wilt disease in avocado has been tested multiple times without a clear consensus. Through an economic analysis, Ploetz et al. (2011) showed that the macro-infusion of propiconazole was not cost-effective for managing laurel wilt in commercial avocado production; therefore, the authors used potted plants to test its efficacy when used as a soil drench and as topical bark application using a bark-penetrating surfactant (Pentra-Bark®; Quest Products Corp., Linwood, KS, USA). A similar study, using the only propiconazole formulation approved for use in commercial avocado trees (Tilt®, Syngenta), was conducted by Ploetz et al. (2017b). Both studies showed that both application methods prevented disease development after artificial inoculation and that topical bark application was more (Ploetz et al. 2011) or equally (Ploetz et al. 2017b) efficacious as the drench. However, authors suggested that topical bark applications would likely only be effective in young trees (< 15 cm diameter) because the thicker bark of mature trees could restrict product penetration. Using mature avocado trees, Crane et al. (2015) tested the efficacy of propiconazole (PropiconazolePro®; Micro Flo Co., Memphis, TN, USA), tebuconazole (Teb #1; Rainbow Treecare, Minnetonka, MN, USA), and thiabendazole (Arbotect® 20-s, Syngenta) delivered as macro-infusion. Authors reported that 60 d after artificial inoculation, none of the 18 propiconazole-treated trees showed external symptoms, while all the nontreated trees and 17% and 44% of trees treated with tebuconazole and thiabendazole, respectively, were symptomatic (Crane et al. 2015). By 239 d after inoculation, a mean of 83% of the propiconazole-treated trees showed symptoms, but the severity (percentage symptomatic canopy) was lower compared with the other treatments (Crane et al. 2015). Based on these results, Crane et al. (2015) recommended the injection (application of concentrated or nondiluted fungicide) or the macro-infusion of propiconazole to prophylactically treat healthy avocado trees near laurel wilt–affected trees (spot treatment). Later, Ploetz et al. (2017b) compared the efficacy of propiconazole in two commercial formulations (Tilt® and PropiconazolePro®) macro-infused in mature trees and found that while both formulations were efficacious in reducing laurel wilt disease severity, PropiconazolePro® provided better control and resulted in trees with highest concentrations of propiconazole in their xylem. In addition, it was reported that macro-infusion was a superior application method compared with injection. The same study found that propiconazole concentration in the xylem of mature trees after bark applications was very low when compared with the concentrations reached by macro-infusion. Regardless of the application method and formulation, it has been reported that propiconazole degradation is temperature dependent, and trees need to be re-treated at least every 12 to 18 months (Armstrong 1999; Crane et al. 2015, 2020; Ploetz et al. 2017b).

Because of the high cost involved in applying fungicides through macro-infusion (~$55 to $66 per tree), this application method has not been adopted by local growers, whereas ~20% of the commercial avocado acreage in south Florida has been injected prophylactically with propiconazole (~$7.5 per tree) on a 12- to 24-month interval (Archer et al. 2022). However, the efficacy of the injection treatment has never been systematically assessed and reports are only anecdotal. Although it is common to find injected trees with laurel wilt symptoms, local growers state that treated orchards present overall fewer trees succumbing to the disease. In cross-sections of propiconazole-injected and laurel wilt–infected trees, it is common to observe that propiconazole is not uniformly distributed in the vascular system, leaving "unprotected" areas that are colonized by the pathogen (Gazis R, unpublished). Therefore, there is a critical need for an alternative fungicide application method that is cheaper and offers a uniform distribution of the a.i. within the tree's vascular system. The objectives of this study were to: 1) evaluate if propiconazole (in the commercial formulation Tilt®) can mobilize from the root system into the main trunk and secondary branches of mature orchard avocado trees when it is applied as a soil drench; 2) test if the concentrations of propiconazole accumulated in secondary branch tissue when propiconazole is applied as a soil drench and trunk injected has an effect on laurel wilt disease development; 3) assess the efficacy of propiconazole in preventing the persistence of the laurel wilt pathogen in stumps of infected trees.

Material and methods

Greenhouse trial – Propiconazole (Tilt®) used as a soil drench in potted trees

PLANT MATERIAL AND PROPICONAZOLE TREATMENTS. 'Lula' (Guatemalan × West Indian) plants were grown from seeds in 3.6-L pots using a commercial potting mix (40% flat peat, 50% pinebark, 10% coarse sand, 10 lb dolomite). Plants were maintained in a shadehouse [average range 15.5 to 31.6 °C, 66% to 82% relative humidity (RH)] at TREC (lat. 25.5°N, long. 80.5°W, elevation 2 m) until they were 16 months old (average 1.7 cm in diameter at the soil level). Plants were pruned to keep only the main stem and transferred 1 month before the trial to a greenhouse (27 to 30 °C, 60% to 70% RH) to allow for acclimatization. A total of 56 'Lula' plants were used in this experiment. This cultivar was selected because it is highly susceptible to laurel wilt, and external symptoms are consistently developed after artificial inoculation. Plants were

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randomly distributed in the greenhouse, and 14 plants were randomly assigned to each of the treatments: 1) 1X treatment, propiconazole basal dose according to the rate recommended on the Tilt® label (3.2 g a.i. per inch tree diameter); 2) 2X treatment, twice the basal dose; 3) 4X treatment, four times the basal dose; and 4) nontreated control plants. The dose for the 1X treatment was calculated based on the average stem diameter of all plants, measured at the base. Based on this average, the amount of propiconazole applied to each plant at the basal dose (1X) was 5 mL per plant (equivalent to 2.1 g a.i.). The dose was divided into two media drench applications, with 2 weeks in between, and the volume of propiconazole per application for each treatment was diluted in 300 mL water per potted plant. The concentration of propiconazole in the solution applied was equivalent to 3595 ppm for the 1X treatment.

Inoculation of potted trees. One month after the second application, 12 plants from each treatment were inoculated with a conidial suspension of *H. lauricola* (strain RL4, specimen CBS 127349) at a final concentration of 5×10^7 conidia/mL. The inoculum was prepared using the protocol described in Navia-Urrutia et al. (2022). Two holes were made in the stem, using a portable drill and a conical drill bit (0.7 × 0.4 cm) at a 45° downward angle. The first hole was made at 8.5 cm above the soil line, and the second at 1.5 cm above the first hole, on the opposite side of the stem. The inoculum was placed in the holes (10 µL per hole) with a micropipette, and the stem was wrapped with parafilm. Inoculated plants were kept under greenhouse conditions (27 to 30 °C, 60% to 70% RH) and monitored for symptom development. Plants were irrigated every other day.

Viability of *H. lauricola* in propiconazole-treated potted trees. To test the viability and multiplication of *H. lauricola* inside the plant's vascular system, stem tissue was collected from three plants per treatment at 5, 11, 15, and 21 d after inoculation (dai). After rating the external symptoms using a 1 to 10 disease severity scale (Navia-Urrutia et al. 2022), the bark was removed to document the presence of internal symptoms (discoloration of the xylem vessels). Disks (~2 mm thick) were excised from the stem with a bypass pruner at different distances from the inoculation points, considering the intermediate point between the two inoculation holes as the "zero" point. From each plant, three disks were excised below the "zero" point (at −2.5, −5.0, and −7.5 cm), and 18 disks were excised above the "zero" point (at 2.5, 5.0, 7.5, 12.5, 17.5, and every 7.5 cm until 115 cm). Stem disks were surface sterilized and placed in petri dishes (100 mm × 15 mm) containing cycloheximide streptomycin malt agar (CSMA*) medium, according to the protocol described in Navia-Urrutia et al. (2022), and incubated at 25 °C for 10 d to recover *H. lauricola* from tissue.

Residue (propiconazole) analysis in drenched potted trees. To test the translocation of propiconazole from the roots into the stem, sapwood samples were collected from two plants per treatment 2 months after the second application. Because of the small diameter of the potted trees, the bark of the entire stem was removed, and slices of the sapwood were collected with a knife. The samples were ground into a fine powder with liquid nitrogen and sent for propiconazole residue analysis through liquid chromatography with tandem mass spectrometry (LC-MS/MS) to AGQ Laboratories (Oxnard, CA, USA). Concentrations of propiconazole measured (in parts per million) are reported in Table 1.

Field trial – Propiconazole used as a soil drench in orchard trees

Plant material, site description, and treatments. Mature 16-year-old avocado trees growing at TREC were used in this experiment. The experimental orchard (experimental plot) consisted of 57 trees distributed in three rows: 41 'Simmonds' (pure West Indian ecotype) and 16 'Beta' (Guatemalan × West Indian) trees. 'Beta' trees were planted every three 'Simmonds' trees. An additional 11 trees in a fourth row (three 'Beta' and eight 'Simmonds') were used as nontreated and noninoculated controls. Scions of the two cultivars were grafted on Waldin (pure West Indian ecotype) rootstock. This plot represents a common south Florida avocado commercial planting, in which a pollinator cultivar (Beta = flower type b) is interplanted within a commercial cultivar (Simmonds = flower type a). One month before the trial, trees were pretreated with mefenoxam (Ridomil Gold® SL, Syngenta) at a rate of 6.2 g a.i. per tree, to promote root health and protect the roots from root rot (*Phytophthora* sp.). The stem diameter of all trees was measured at the base, and the average was calculated for the entire plot to determine the basal dose of propiconazole according to the rate recommended on the Tilt® label (3.2 g a.i. per inch tree diameter). Four treatments were evaluated: 1) 1X treatment, a basal dose of propiconazole at 25.5 g a.i. per tree; 2) 2X treatment (twice the basal dose); 3) 4X treatment (four times the basal dose); and 4) nontreated control. The experiment was set up in a randomized complete block design (RCBD), where each field row was considered a block. In each block, four or five trees were assigned to each of the four treatments, depending on the total number of trees available in the row. Four trees were assigned to each treatment in the middle row, whereas five trees were assigned to each treatment in the external rows (except for six trees assigned to the 4X treatment in the eastern row). The dose of propiconazole corresponding to each treatment was divided into two applications with 2 weeks in between. For each application and tree, the corresponding volume of propiconazole was dissolved in 5 gal of water and applied directly to the soil using an air blast sprayer (Rears Pulblast Sprayer; Chemical Containers Inc., Lake Wales, FL, USA) outfitted with a handgun (30 psi) covering an area of ~11.2 m^2 around the trunk. The concentration of propiconazole in the 1X solution was 674 ppm. An additional trial to evaluate the absorption and translocation of propiconazole to vegetative and fruit tissue was conducted in a separate plot (residue plot) containing 17-year-old 'Choquette' (Guatemalan × West Indian) trees grafted on 'Waldin' (West Indian ecotype) rootstock. This cultivar was selected because the fruit-bearing time coincided with the first propiconazole application, while the cultivars in the experimental plot had already finished fruiting. The plot consisted of 16 trees, which were also treated with mefenoxam as reported previously, and four trees were randomly assigned to each of the four treatments reported for the experimental plot. Soil applications of propiconazole were made on the same dates in both plots.

Table 1. Concentration of propiconazole measured in the sapwood from different tissues of potted (greenhouse) and orchard avocado trees (residue and experimental plots) drenched with three rates of propiconazole (Tilt®; Syngenta, Greensboro, NC, USA) and from propiconazole-injected trees (commercial plot). In the drench trials, the plants used to measure the concentration of propiconazole in tissue were randomly selected from the total number of plants in each trial (56 plants in the greenhouse trial, 16 and 57 trees in the residue and experimental plot, respectively), whereas the total number of plants in the injected commercial plot (10 trees) were analyzed.

Cultivar/tree no.	Application method and rate of propiconazole[i]	Propiconazole concn measured in the sapwood from different tissues (ppm)[ii]		
		Secondary branch	Root flare	Stem
		Potted trees (greenhouse)		
Lula/tree 1	Soil drench - 1X	ND[iii]	ND	2.68
Lula/tree 2	Soil drench - 1X	ND	ND	6.56
Lula/tree 3	Soil drench - 2X	ND	ND	4.00
Lula/tree 4	Soil drench - 2X	ND	ND	5.44
Lula/tree 5	Soil drench - 4X	ND	ND	4.84
Lula/tree 6	Soil drench - 4X	ND	ND	7.24
		Residue plot[iv]		
Choquette	Nontreated control	0.060	<0.010	ND
Choquette/tree 2	Soil drench - 1X	0.032	<0.010	ND
Choquette/tree 8	Soil drench - 2X	0.120	0.360	ND
Choquette/tree 6	Soil drench - 4X	0.104	0.290	ND
		Experimental plot[iv]		
Simmonds/tree 17–24	Nontreated control	0.020	0.012	ND
Simmonds/tree 18–28	Nontreated control	<0.010	<0.010	ND
Simmonds/tree 16–18	Soil drench - 1X	0.040	0.076	ND
Beta/tree 18–15	Soil drench - 1X	0.024	0.044	ND
Simmonds/tree 15–18	Soil drench - 2X	0.024	0.480	ND
Simmonds/tree 17–28	Soil drench - 2X	0.020	0.192	ND
Simmonds/tree 15–9	Soil drench - 4X	0.028	0.848	ND
Simmonds/tree 16–4	Soil drench - 4X	0.024	0.148	ND
		Experimental plot[v]		
Simmonds/tree 15–20	Nontreated control	<0.010	ND	ND
Simmonds/tree 15–8	Soil drench - 1X	<0.010	ND	ND
Simmonds/tree 17–10	Soil drench - 1X	<0.010	ND	ND
Simmonds/tree 15–17	Soil drench - 2X	<0.010	ND	ND
Simmonds/tree 17–30	Soil drench - 2X	0.012	ND	ND
Simmonds/tree 15–9	Soil drench - 4X	0.016	ND	ND
Beta/tree 17–15	Soil drench - 4X	0.012	ND	ND
		Commercial plot[vi]		
Buck 3/tree 1	Injection - 1X	<0.010	ND	0.024
Buck 3/tree 2	Injection - 1X	0.472	ND	0.020
Buck 3/tree 3	Injection - 1X	14.5	ND	32.0
Buck 3/tree 4	Injection - 1X	21.0	ND	0.180
Buck 3/tree 5	Injection - 1X	294.0	ND	0.010
Buck 3/tree 6	Injection - 1X	38.0	ND	0.224
Buck 3/tree 7	Injection - 1X	0.036	ND	<0.010
Buck 3/tree 8	Injection - 1X	1.020	ND	0.012
Buck 3/tree 9	Injection - 1X	0.212	ND	<0.010
Buck 3/tree 10	Injection - 1X	0.060	ND	0.400

[i] Propiconazole rate: 1X represents the rate recommended on the Tilt® label (3.2 g a.i. per inch diameter), 2X and 4X twice and four times the recommended rate, respectively; 1 g/inch = 0.3937 g·cm^{-1} = 0.0353 oz/inch.

[ii] Concentration measured by liquid chromatography with tandem mass spectrometry (LC-MS/MS), limit of quantification of the analysis = 0.010 ppm. The level of uncertainty for propiconazole in the analysis is 32.6% (AGQ Laboratories, Oxnard, CA, USA); 1 ppm = 1 mg·kg^{-1}.

[iii] ND = no data. Propiconazole concentration was not measured in that specific tissue.

[iv] Samples from secondary branches and root flares of trees in the residue plot were taken 1 month after the application of propiconazole, samples from the experimental plot were taken 2 and 3 months after the application.

[v] Samples from inoculated secondary branches of trees in the experimental plot were taken 4 weeks after inoculation.

[vi] Samples from secondary branches of injected trees were taken 3 weeks before their artificial inoculation, and from the fork under the inoculated branch (stem) 15 weeks after inoculation.

Residue (propiconazole) analysis in drenched orchard trees. 'Choquette' trees in the residue plot were tested for propiconazole residue 1 month after the second propiconazole application. Physiologically mature fruit and sapwood samples taken from root flares and from primary and secondary branches were collected from one tree under each treatment. To collect sapwood samples from vegetative tissue, three openings were made in the bark with a hole saw

drill bit (2 1/8 inch), and after the bark was removed, sapwood sawdust was obtained by drilling four to five holes, ~1 cm deep, with a spade drill bit (3/4 inch). Sapwood samples from each tissue were mixed, ground into a fine powder with liquid nitrogen, and sent for propiconazole residue analysis (LC-MS/MS) to AGQ Laboratories, together with fruit. In the experimental plot, two randomly selected trees from each treatment were tested for propiconazole residue in sapwood tissue collected from root flares and secondary branches, 2 and 3 months after the second application of propiconazole. In addition, to test the degradation of the a.i. inside the vascular tissue, sapwood samples from inoculated branches were sent for analysis 1 month after the artificial inoculation (4.5 months after the second application). Sapwood samples were collected and analyzed as previously described. Concentrations of propiconazole measured (in parts per million) are reported in Table 1.

Artificial inoculation of drenched orchard trees. 'Simmonds' and 'Beta' trees in the experimental plot were inoculated in Jan 2022 (3.5 months after the second application of propiconazole) with a conidial suspension of *H. lauricola* (strain RL4) at a final concentration of 1.2×10^5 conidia/mL. The inoculum was prepared using the protocol described in Navia-Urrutia et al. (2022). Six holes were made in a secondary branch using a 3/32-inch drill bit. In each hole, 25 μL of inoculum was placed with a micropipette (150 μL total), and the branch was wrapped with stretch plastic film.

Evaluation of symptom development, removal of infected branches, and "stumping." Evaluation of symptom development on inoculated branches began 1 week after inoculation. The number of trees with initial (loss of turgor on leaves, "green-wilting") or advanced external symptoms (leaves showing desiccation, "brown wilting") in the inoculated branch was recorded every 2 or 3 d up to 1 month after inoculation. The disease incidence was calculated for each treatment in each block as the percentage of symptomatic trees in each treatment per row.

To test if the application of propiconazole as a soil drench and the removal of the symptomatic branches, a common management practice implemented by Florida's avocado growers, could restrict the movement of the pathogen to the noninfected branches, the inoculated branches were cut (1 ft under the bifurcation or tree fork) as soon as the symptoms were conspicuous (brown wilting), or 1 month after inoculation, regardless of symptoms. Because the inoculated branch was cut, disease severity was not recorded in our experiment. Development of symptoms in noninoculated branches was recorded weekly from weeks 3 to 8 after inoculation and then every 4 or 5 weeks until week 34 (~8 months after inoculation).

To evaluate the potential synergistic effect of propiconazole drenching and tree "stumping" on the health of the stump and re-sprouts, trees were cut to a stump (~4 ft) when a noninoculated branch showed advanced symptoms (brown wilting and/or defoliation). Stumps were "whitewashed" with 50/50 white latex paint/water to prevent sunburn. The number of stumped trees with re-sprouts was recorded, as well as the development of laurel wilt symptoms in the new stems. The presence of internal symptoms in the stumps (discoloration of the xylem vessels) was evaluated 8 months after inoculation. To assess the viability of the *H. lauricola*, xylem tissue was removed from 10 stumps that were chosen randomly, surface disinfected, and placed on CSMA* media (Navia-Urrutia et al. 2022).

Data analysis. Normality and homogeneity of variances were assessed using the Shapiro-Wilk test and the Levene test, respectively. Data from disease incidence were subjected to a two-way analysis of variance using the general linear model. Row effects were not detected, and disease incidence means were separated using Tukey's honestly significant difference test at a 5% level of significance. All data were analyzed using SAS Studio (version 3.8) on SAS (version 9.4; SAS Institute Inc., Cary, NC, USA).

Field trial – Propiconazole injection in trees in a commercial orchard

Plant material and propiconazole treatment. Ten mature orchard 'Buck 3' (Guatemalan × West Indian) trees, grafted in 2013 on 'Waldin' rootstocks, were donated by a commercial producer. This cultivar was chosen because it is a late cultivar, having harvestable fruit until the end of May, which overlapped with our experiment. Trees were injected with propiconazole (Tilt®) five times, from Jan 2014 to Jun 2021, using a modified version of the no-drill tree trunk injection system (Wedgle® Direct-Inject™; Arbor Systems, Omaha, NE, USA). Trees received the propiconazole dose according to the rate recommended on the Tilt® label (3.2 g a.i. per inch tree diameter), with 16 to 23 months between each injection. The 10 trees had similar architecture, including the number of secondary branches and canopy cover. The commercial plot contained other trees injected with propiconazole but not artificially inoculated with the pathogen, which served as controls.

Residue (propiconazole) analysis, inoculation, and evaluation of symptom development. Three weeks before inoculating the trees, sapwood samples taken from one side of a secondary branch in each of the 10 trees were sent for propiconazole residue analysis, as previously described. In May 2022, the opposite side of the same branch was inoculated with *H. lauricola*, following the same procedures as in the experimental plot. Development of external symptoms was monitored weekly up to 3 months after inoculation. The presence of internal symptoms and viability of *H. lauricola* were evaluated 1 month after inoculation by removing a piece of bark and sampling xylem tissue at least 10 cm below the inoculation points. Xylem tissue collected was surface disinfected and placed on semiselective media as described before. The advancement of internal symptoms was evaluated 3.5 months after the inoculation on the trunk, adjacent branches, and root flares. Sapwood samples from the fork under the inoculated branch were collected as previously described, and sent for residue analysis together with fruit from two trees with the highest propiconazole concentration in the inoculated branch. Concentrations of propiconazole measured (in parts per million) in vegetative tissue before and after inoculation are reported in Table 1. Trees were cut to a stump (~4 ft) in Sep 2022, and a final evaluation of internal symptoms was conducted in Jan 2023, as previously described.

Results

Greenhouse trial – Propiconazole used as a soil drench in potted trees

Translocation of propiconazole in potted trees. At the low dose of propiconazole used (1X), the concentration

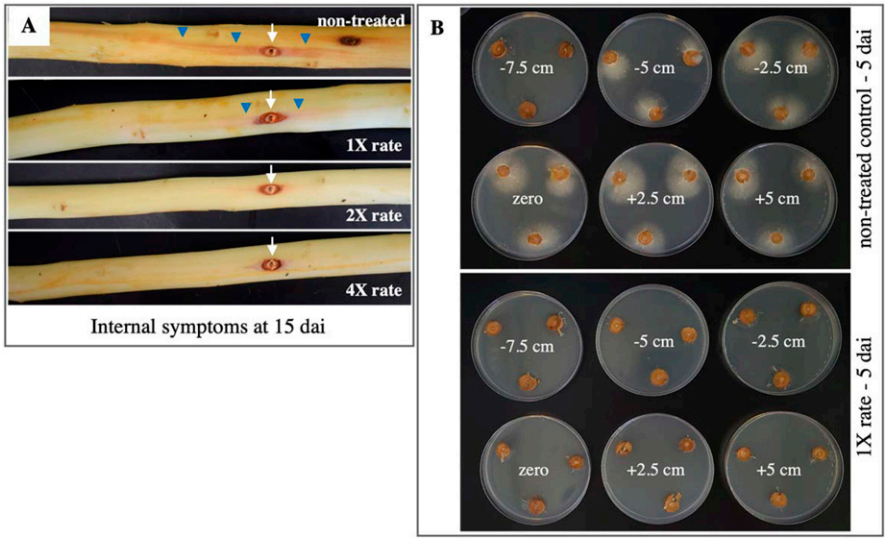

Fig. 1. Laurel wilt internal symptoms and recovery of *Harringtonia lauricola* from potted avocado plants drenched with different rates of propiconazole [1X: rate recommended in the Tilt® (Syngenta, Greensboro, NC, USA) label (3.2 g a.i. per inch diameter), 2X: twice the rate recommended, 4X: four times the rate recommended] or nontreated. (A) Discoloration of xylem vessels in potted plants 15 d after inoculation (dai). Characteristic streaking (pointed with blue triangles) coming out from the inoculation point (white arrow) is observed in nontreated control plants and 1X propiconazole-treated plants. (B) Recovery of *H. lauricola* from samples taken 5 dai at different distances from the inoculation point (up to 7.5 cm below and 5 cm above the zero point) and incubated in semiselective media cycloheximide streptomycin malt agar (CSMA*) for 11 d. *H. lauricola* colonies growing from stem disks were only recovered from nontreated plants, no growth was observed from the disks of propiconazole-treated plants (disks from 2X or 4X rates were not included in the figure); 1 g/inch = 0.3937 g·cm^{-1} = 0.0353 oz/inch, 1 cm = 0.3937 inch.

of propiconazole drenched in 600 mL was equivalent to 3595 ppm. The concentration of propiconazole measured in the sapwood samples ranged from 2.68 to 7.24 ppm, indicating that although propiconazole can be translocated from the roots to the stems of young plants, much of the a.i. is not assimilated. No association was observed between the amount applied (1X, 2X, or 4X) and the concentration measured in the tissue (Table 1).

Development of symptoms in potted trees and *H. lauricola* viability. Petiole bending (first symptom of drought stress) in control nontreated plants started at 10 dai and by the end of the evaluation period (21 dai), 70% to 100% of leaves were wilted. Discoloration (streaking) of xylem vessels was first observed in nontreated plants at 11 dai, and by 15 and 21 dai, the entire length of the stem was discolored. External symptoms were not observed in propiconazole-treated plants. Vascular discoloration was observed in 1X-treated plants, but only at 15 and 21 dai, and was limited to 20 cm above the inoculation point. Vascular damage was not observed in 2X or in 4X-treated plants (Fig. 1A). *Harringtonia lauricola* was only recovered from samples taken from nontreated plants. Colonies were recovered up to 32.5 cm above and 5 cm below the "zero" point at 5 dai (Fig. 1B). By 11 dai, colonies were recovered from stem discs taken at 100 cm above and 7.5 cm below the "zero" point. By 15 and 21 dai, colonies were recovered from all the samples, up to 115 cm above and 7.5 cm below the "zero" point.

Field trial – Propiconazole used as a drench in orchard trees

Translocation of propiconazole in orchard trees. At the low dose of propiconazole, the concentration of a.i. used to drench trees (in 10 gal) was equivalent to 674 ppm propiconazole. The concentration of propiconazole in samples collected in the residue and in the experimental plot confirmed the translocation of propiconazole from roots to branches (Table 1); however, the concentrations were below the fungicidal threshold [> 1 ppm (Mayfield et al. 2008)]. Effective concentrations to cause 100% inhibition growth (EC_{100}) of *H. lauricola* [> 0.1 ppm (Mayfield et al. 2008)] were only present in the root flares of trees under 2X and 4X treatments (Table 1). No association was observed between the dosage of propiconazole applied to the soil and the concentration of propiconazole measured in the tissues. Propiconazole was not detected in fruit (data not shown).

Development of symptoms on inoculated branches. Inoculated branches started to show early laurel wilt symptoms ("green-wilting") 15 dai, regardless of the treatment. Three days later (18 dai), advanced symptoms, including leaf desiccation ("brown-wilting") and wilting of the flowers were observed in several trees. By 25 dai, inoculated branches in all the trees presented symptoms (early or advanced) except two trees in the 4X treatment. By 27 dai, disease incidence was 100% in all the treatments (Table 2). Statistically significant differences in mean disease incidence between the treatments were not detected on any of the days evaluated. Advanced symptoms were observed in all the inoculated branches by 32 dai. Symptoms were not observed in any of the 11 nontreated and noninoculated control trees.

Development of symptoms on noninoculated branches. Symptom development in noninoculated branches was evaluated after the removal of the inoculated branch. By 35 dai, all treatments had trees showing early laurel wilt symptoms in noninoculated branches. All the nontreated control trees (n = 14) showed symptoms in noninoculated branches 49 dai, whereas several trees from the propiconazole-treated treatments remained asymptomatic (Table 3). By 91 dai, only ~40% of the trees in the 4X treatment showed symptoms in noninoculated branches. Differences in disease incidence mean between the 4X treatment and other treatments were significant at 35 dai, and from 49 up to 119 dai (Table 3). Because of this delay in symptom development, in comparison with the other treatments, tissue samples from the 4X treatment trees were sent for propiconazole residue analysis. Concentrations of propiconazole in all

Table 2. Effect of propiconazole (Tilt®; Syngenta, Greensboro, NC, USA) drenched at different rates on the percentage of orchard avocado trees with laurel wilt disease symptoms (disease incidence) in the inoculated branch, evaluated from 15 to 27 d after inoculation (dai).

	Percentage of trees with laurel wilt disease symptoms in inoculated branches over the time (mean ± *SD*)					
Propiconazole rate[i]	15 dai	18 dai	20 dai	22 dai	25 dai	27 dai
1X	61.67 (± 37.53)	76.67 (± 25.17)	91.67 (± 14.43)	100.00	100.00	100.00
2X	21.67 (± 2.89)	55.0 (± 27.84)	78.33 (± 2.89)	91.67 (± 14.43)	100.00	100.00
4X	20.53 (± 4.23)	56.1 (± 29.35)	72.77 (± 11.81)	81.1 (± 20.09)	86.67 (± 23.09)	100.00
Nontreated control	23.33 (± 25.17)	78.33 (± 20.21)	91.67 (± 14.43)	100.00	100.00	100.00
Significance[ii]	NS	NS	NS	NS	NS	NS

[i] Propiconazole rates used in the treatments: 1X represents the rate recommended on the Tilt® label (3.2 g a.i. per inch diameter), 2X and 4X are twice and four times the recommended rate, respectively; 1 g/inch = 0.3937 g·cm^{-1} = 0.0353 oz/inch.

[ii] Significance between treatments is indicated within columns for each evaluation day; NS = nonsignificant at $P < 0.05$.

trees (n = 15) were below the fungistatic threshold [EC_{100} = 0.1 ppm (Mayfield et al. 2008)], ranging from < 0.010 to 0.056 ppm, and no association was observed between the concentration of propiconazole in the sample and the presence of symptoms (data not shown). By the end of the evaluation (almost 8 months after inoculation), three trees from the 4X treatment, two from the 2X treatment, and two from the 1X treatment remained asymptomatic.

Development of symptoms in tree stumps and new shoots. Ninety-eight percent of the trees cut to a stump produced new shoots. External symptoms, ranging from green wilting to dieback and defoliation, were observed in 28% and 40% of the stump resprouts at 4 and 5 months after trees were cut, respectively. Internal symptoms were evaluated 5 months after the trees were cut. The production of a new ring of xylem, apparently healthy (without streaking), was observed in 34% of the stumps, most of which were holding healthy canopies (Fig. 2A and B). New xylem with some degree of streaking (Fig. 2D) or completely discolored was present in 66% of the stumps, holding a range of healthy and symptomatic canopies (Fig. 2C). The old xylem was severely damaged in 100% of the stumps, regardless of whether they sprout or hold a healthy or symptomatic canopy, and regardless of the propiconazole treatment applied. To test the presence of *H. lauricola* in the new and old xylem rings, samples were collected from 10 stumps. The pathogen was recovered from six of eight samples taken from apparently healthy new xylem, and from one of two samples taken from symptomatic new xylem. The pathogen was recovered from all old xylem samples.

Field trial – Propiconazole injection in trees in a commercial orchard

Propiconazole residue analysis. Concentrations of propiconazole in secondary branches of injected trees were highly variable, ranging from < 0.010 to 294 ppm (Table 1). Concentrations in the trunk, taken under the fork of the inoculated branch 4 months later were also variable but, in general, lower than in the branches (Table 1). Propiconazole was not found in fruit tissue taken from trees number 5 (294 ppm) and 6 (38 ppm) (data not shown), in which the highest concentrations of propiconazole in the secondary branches were detected.

Development of symptoms on inoculated and noninoculated branches. Two weeks after inoculation, out of 10 trees, two showed early (trees 1 and 2) and two showed advanced (trees 8 and 9) laurel wilt symptoms. Four weeks after inoculation, nine of the 10 trees showed advanced symptoms, regardless of the propiconazole concentration present in the inoculated branch. Tree number 7 (0.036 ppm) remained asymptomatic until the end of the evaluation (15 weeks after inoculation). Five weeks after inoculation, internal damage of the xylem vessels was confirmed above and below the inoculation points in all trees. In tree number 7, vascular damage was limited to the area close to the inoculation points. *H. lauricola* colonies were recovered from sapwood samples taken under the inoculation points from all the trees. Trees number 8 (1.02 ppm), 5 (294 ppm), and 3 (14.5 ppm) showed external symptoms in noninoculated branches at 5, 9, and 15 weeks after inoculation, respectively. Evaluation of internal symptoms 15 weeks after inoculation revealed that the pathogen had colonized the tissue under the inoculated branch fork in tree numbers 3, 5, and 8, and vascular damage was observed in the root flares of trees 5 and 8. After 8 months from inoculation, vascular damage was observed in all stumps except those from trees 7 and 9, and *H. lauricola* was recovered from four of eight stumps (tree numbers 1, 3, 5, and 10) with internal symptoms.

Discussion

Current strategies to manage laurel wilt disease are limited and rely on implementing cultural practices, including sanitation, pruning (light management), and preventive use of propiconazole (Crane et al. 2020; Olatinwo et al. 2021). The prophylactic injection of propiconazole was recommended to protect healthy trees near a laurel wilt outbreak (spot treatment) or the entire orchard (Crane et al. 2016, 2020). Currently, in south Florida, USA, propiconazole injections are used in ~20% of the commercial avocado acreage (Archer et al. 2022). However, before this study, its effectiveness in controlling the pathogen under field conditions had not been systematically tested. In addition, this study responded to a critical need to assess a less costly application method that could homogeneously deliver propiconazole within the tree's vascular system.

Results from the greenhouse assay showed that propiconazole, used as a drench, effectively controlled the multiplication of *H. lauricola* and prevented the development of laurel wilt symptoms. Drench applications of propiconazole (in the commercial formulations Alamo® and Tilt®) to potted plants were previously reported as efficacious by Ploetz et al. (2011), but external and internal symptoms were observed in some of the replications,

Table 3. Effect of propiconazole (Tilt®; Syngenta, Greensboro, NC, USA) drenched at different rates on the percentage of orchard avocado trees with laurel wilt disease symptoms (incidence) in noninoculated branches, evaluated from 35 to 238 d after inoculation (dai).

	Percentage of trees with laurel wilt disease symptoms in noninoculated branches over the timeline (mean ± *SD*)									
Propiconazole rate[i]	35 dai	42 dai	49 dai	56 dai	91 dai	119 dai	154 dai	189 dai	210 dai	238 dai
1X	53.33 (± 41.63) ab[ii]	60 (± 34.64)	66.67 (± 30.55) ab	66.67 (± 30.55) ab	73.33 (± 23.09) a	86.67 (± 11.55) a	86.67 (± 11.55)	86.67 (± 11.55)	86.67 (± 11.55)	86.67 (± 11.55)
2X	58.33 (± 17.56) ab	65.0 (±21.79)	65.0 (± 21.79) ab	80.00 (± 20.00) ab	86.67 (± 11.55) a	86.67 (± 11.55) a	86.67 (± 11.55)	86.67 (± 11.55)	86.67 (± 11.55)	86.67 (± 11.55)
4X	0.00 b	41.11 (± 8.39)	41.11 (± 8.39) b	41.11 (± 8.39) b	41.11 (± 8.39) b	58.89 (± 8.39) b	64.44 (± 17.10)	70 (± 26.46)	78.33 (± 20.21)	78.33 (± 20.21)
Nontreated control	73.33 (± 30.55) a	93.33 (± 11.55)	100.00 a	100.00 a	100.00 a	100.00 a	100.00	100.00	100.00	100.00
Significance[iii]	*	NS	*	*	*	*	NS	NS	NS	NS

[i] Propiconazole rates used in the treatments: 1X represents the rate recommended on the label (3.2 g a.i. per inch diameter), 2X and 4X are twice and four times the recommended rate, respectively; 1 g/inch = 0.3937 g·cm^{-1} = 0.0353 oz/inch.

[ii] Incidence means within columns followed by the same letter are not significantly different based on Tukey's honestly significant difference test at $P < 0.05$.

[iii] Significance between treatments is indicated within columns for each evaluation day; NS, * = nonsignificant and significant at $P < 0.05$, respectively.

and the pathogen was recovered from the vascular tissue of treated plants. Conversely, we did not recover *H. lauricola* from any of the propiconazole-treated potted plants, suggesting that the concentrations found in the vascular tissue (ranging from 2.68 to 7.24 ppm) were sufficient to kill the pathogen. Unfortunately, it is not possible to compare our results with those reported by Ploetz et al. (2011) because propiconazole concentration in the vascular tissue was not determined in the latter. The use of younger plants with a smaller stem diameter (average of 16.8 mm), in comparison with those used by Ploetz et al. (2011) (25-mm diameter), could explain the discrepancies between studies. In young or small-diameter plants, the systemic fungicide and the pathogen are spatially limited to the functional xylem ring, whereas in older and thicker plants, the pathogen may avoid contact with the fungicide by moving and colonizing older xylem rings. Although fungitoxic concentrations of propiconazole have not been determined in planta, results from our greenhouse assays agree with the in vitro results reported by Mayfield et al. (2008), who found that concentrations above 1 ppm were fungitoxic to *H. lauricola*.

Propiconazole residue analysis confirmed that 1 month after the second application of propiconazole to orchard trees, the a.i. was translocated from the roots to the stem. However, the amounts translocated to above-ground tissue were mostly below the in vitro growth inhibition threshold (EC_{100} = 0.1 ppm), and none of the trees accumulated fungitoxic concentrations [> 1 ppm (Mayfield et al. 2008)]. The vascular tissue that contained enough propiconazole to inhibit the multiplication of *H. lauricola* included the root flares of trees under the 2X and 4X treatments in the residue and experimental plots and the secondary branches of trees under 2X and 4X treatments in the residue plot. There are no previous studies documenting the accumulation of propiconazole in field-drenched avocado trees, except for an unpublished account in Ploetz et al. (2017b) stating that propiconazole was not detected in the xylem of 30-year-old avocado trees drenched with propiconazole. Although our results proved the acropetal movement of propiconazole in field avocado trees, the concentrations of propiconazole in the secondary branches [measured 2 and 3 months

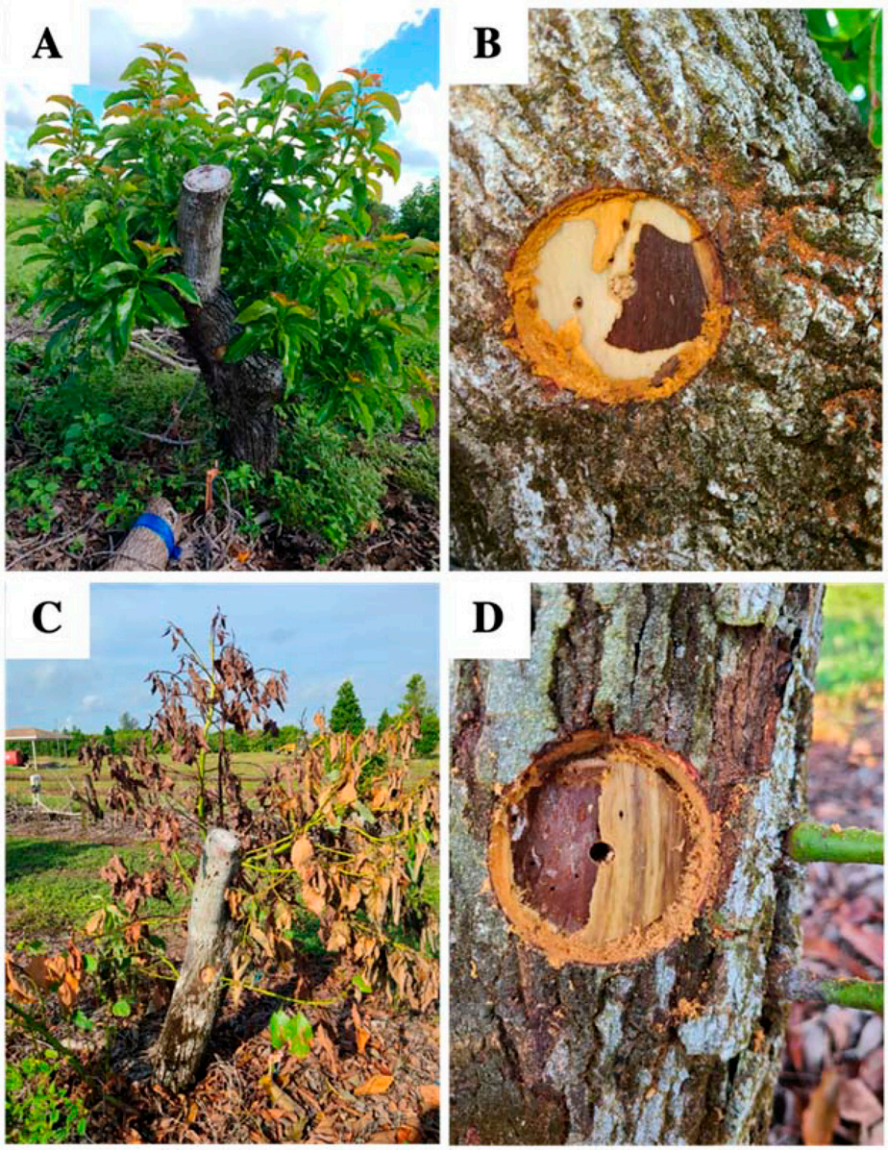

Fig. 2. Avocado stumps and new shoots showing laurel wilt external and internal symptoms. (A) Healthy canopy coming from a stump where the new xylem ring remains asymptomatic (B). (C) Wilted canopy coming from a stump where the new xylem ring has vascular damage (D). The old xylem was severely damaged in both stumps (B) and (D).

after the soil application (pre-inoculation), and 4 and 5 months after the soil application (post-inoculation)] were very low or under the limit of detection (< 0.010 ppm). As expected, our results showed that the concentrations of propiconazole present in the aboveground tissue were not sufficient to inhibit the multiplication of the pathogen in the inoculated branches nor prevent its movement to noninoculated branches. Moreover, the delay in the development of symptoms in the noninoculated branches from trees in the 4X treatment could not be associated with the propiconazole concentration present in the tissue, because trees that remained asymptomatic had concentrations as low as the ones present in the symptomatic trees. Overall, results suggest that a single drench application of propiconazole is not enough to have an effect on laurel wilt development in field avocado trees, which is the opposite of what we observed in potted trees, and that synergistic cultural practices, such as the removal of the symptomatic branch at symptom onset, did not prevent the movement of the pathogen to noninoculated branches in trees under any propiconazole treatment.

It is unknown if recurrent drench applications could favor the accumulation of propiconazole in the below- and above-ground vascular tissue and thus confer protection against natural infections or prevent root-to-root transmission. Previous studies on injected or infused oaks [northern red oak (*Quercus rubra*) northern pin oak (*Quercus ellipsoidalis*)], American elm (*Ulmus americana*), redbay, and avocado have shown that propiconazole degrades and loses its effectiveness in ~1 to 3 years (Armstrong 1999; Blaedow et al. 2010; Crane et al. 2015; Mayfield et al. 2008; Osterbauer and French 1992). Considering this, the low assimilation of the product through the roots, and the type of soils found in south Florida, USA (rapid drainage and subjected to high rainfall), this alternative methodology would probably require more frequent applications or higher volumes of the product per application. Therefore, the use of propiconazole as a soil drench would not be cost-effective and not legal (maximum application 7.09 lb/acre a.i. per year). It is also unknown if applications at higher doses or frequency could negatively influence the soil microbiota in avocado commercial plantations. In a recent publication, Roman et al. (2021) reported the negative effect of extended applications of propiconazole on soil microbiota and its function.

Contrary to our findings, the use of propiconazole as a drench has proven to be effective in controlling other tree diseases under field conditions. For example, Wilson and Lester (1996) reported that the application of propiconazole to southern live oaks (*Quercus virginiana*) through the dripline, provided protection against the oak wilt pathogen up to 2 years after treatment. In a later study, Wilson and Lester (2002) reported low levels of oak wilt disease incidence in oak trees treated with propiconazole using macroinjections, microinjections, or soil application to the dripline, indistinct of the application method. More recently, Watpade et al. (2022) reported that potted and field-planted apple (*Malus ×domestica*) seedlings, artificially inoculated with the white root rot pathogen (*Dematophora necatrix*) and soil drenched with propiconazole, remained symptomless up to 3 years after the treatment.

Cutting trees to stumps (~4 ft, called "stumping") is a common practice to rejuvenate mature avocado trees. This technique promotes the generation of vigorous new sprouts, which may

resume fruit production within 3 years. In addition, a different cultivar can be grafted on the resprouts, reducing the time of fruit production (Hofshi et al. 2010). In south Florida, laurel wilt-infected trees are often "stumped" and maintained in the field to regrow the canopy or for top-working (i.e., grafting to a new cultivar). According to Crane et al. (2020), growers' accounts of the outcome of these trees are contradictory, with some reporting stumps with healthy canopies that become productive after 2 to 3 years, and others reporting symptomatic canopies that eventually succumb to the disease. The evaluation of the "stumped" trees in our experimental plots (drenched and injected) showed that after the tree is infected, the pathogen remains viable in the old rings of the xylem and can move and infect new rings as these are produced, compromising the health of the new sprouts and eliminating the possibility of using the stumps for future grafts. How fast the pathogen infects the new xylem is unknown; some stumps in our experimental plots presented asymptomatic new xylem rings, whereas in others, the new xylem had symptoms of vascular damage ("streaking"). Pathogen titer in the stumps, influenced by how quickly the tree was "stumped" after symptom onset, likely determines the health of the new xylem ring. Variations in stumping time could explain growers' conflicting observations. Our study supports the recommendation that laurel wilt–infected trees must be completely removed from the planting (Crane et al. 2020) because the pathogen cannot only move upward and reinfect the new canopy, but infected stumps represent a source of inoculum for adjacent trees because of root-to-root transmission.

The concentrations of propiconazole in field injected trees were highly variable, ranging from < 0.010 to 294 ppm. Because all the trees in that commercial plot received the same number of injections and dosage, the residue analysis confirmed the heterogeneous distribution of the fungicide within and among trees. The uneven distribution of propiconazole was also reported in injected red oaks (Blaedow et al. 2010). The authors confirmed the acropetal and basipetal movement of propiconazole but reported high concentration variability across samples taken at the same time from different trees and from stems and roots of the same tree. The distribution of propiconazole in the crown of elm trees was found to be more uniform when the product was applied through macro-infusion vs. injection (Haugen and Stennes 1999). The same was reported for sassafras (*Sassafras albidum*) trees (Johnson et al. 2023) and field avocado trees (Crane et al. 2016; Ploetz et al. 2017b); however, the use of macro-infusion in commercial avocado production in south Florida is cost-prohibitive (Ploetz et al. 2011).

With the exception of tree number 7 (propiconazole: 0.036 ppm), which did not develop external symptoms, we did not find differences in symptom development among propiconazole-injected trees. Even in tree numbers 3, 5, and 8, with propiconazole concentrations above 1 ppm, reported as fungitoxic in the in vitro assays (Mayfield et al. 2008), the movement of the pathogen to noninoculated branches was observed. Previous studies that tested the efficacy of propiconazole using macro-infusion and injection reported a reduction in cumulative severity or a delay in the development of laurel wilt symptoms over time, but not the absence of symptoms (Crane et al. 2015; Ploetz et al. 2017b). Similarly, noninoculated branches in injected trees developed symptoms later than drenched or nontreated trees, and a general slower disease progress was observed in injected trees. Injected tree number 7, although it did not show external symptoms, presented vascular discoloration in the area surrounding the inoculation points and the pathogen was recovered from the tissue collected ~5 cm away from the inoculation points, confirming that the inoculation method was successful.

In red oaks, the causal agent of oak wilt was recovered from vascular tissue with high concentrations of propiconazole (tens to hundreds of parts per million) 24 months after the trees were injected, but the pathogen was not recovered 2 and 12 months after injection (Blaedow et al. 2010). The authors hypothesized that the reduction in pathogen control was due to the lack of movement of the fungicide to the newly formed xylem rings, and the subsequent movement of the pathogen into unprotected rings. Similarly, we recovered *H. lauricola* from all the inoculated branches, even from tissue containing tens to hundreds of ppm of propiconazole. If the *H. lauricola* inoculum was able to colonize and multiply within the outermost and unprotected xylem rings of propiconazole-injected trees, it would imply that trees should be injected more frequently than every 11 months, because, according to the records, the trees were last injected in Jun 2021 and artificially inoculated on May 2022. In previous experiments in which propiconazole was delivered into field avocado trees through macroinfusion or injection, 1 to 3 months before artificial inoculation with *H. lauricola*, the authors reported an increase in disease incidence and severity 200 dai (Crane et al. 2015; Ploetz et al. 2017b). These results would support the hypothesis that the pathogen can reach and colonize newly formed layers of the xylem, therefore suggesting that the trees should be injected more frequently. Unfortunately, how often avocado trees form a xylem ring in subtropical areas, such as south Florida, is unknown. Alternatively, based on the observed uneven distribution of propiconazole when injected, we hypothesize that although high concentrations of propiconazole were detected in the surrounding sapwood (the side of the branch from where the tissue was collected for residue analysis), the lack of uniform distribution of the compound allowed the pathogen to spread and multiply.

The lack of uniformity in the distribution of injected propiconazole within avocado trees imposes a major complication in the control of *H. lauricola* under natural infestations. Ambrosia beetle galleries, containing symbiotic fungal gardens, extend longitudinally and transversely, reaching as far as the heartwood, which potentially results in a constant release of inoculum into the tree's vascular system. In addition, under natural infestations, trees are often infested by more than one female ambrosia beetle (Hughes et al. 2015). If, as suggested by Blaedow et al. (2010), propiconazole is not mobilized to the new xylem generated after the trees were injected and is not evenly distributed in the active xylem at the injection time, many untreated areas may allow for the multiplication and spread of the fungus. Our observations of the pathogen movement from inoculated branches with high

concentrations of propiconazole to noninoculated branches suggest the presence of areas with propiconazole concentrations below the growth inhibition threshold, allowing the fungus to move across the old and new xylem rings. The mechanisms by which the fungus moves through old and new xylem are beyond the scope of this study, but the lack of effectiveness of propiconazole injections to control laurel wilt under natural conditions should be reconsidered.

In conclusion, although the trials were conducted only once because of the logistics involved in procuring mature orchard trees, our results clearly showed that propiconazole injections do not prevent infection or symptom development and that root absorption of propiconazole and translocation to above-ground tissue in soil drench application is minimal in orchard trees. Systemic fungicides have proven to be effective in controlling a myriad of vascular pathogens; however, because of the poor uptake, the limited movement of propiconazole in the formulation tested inside mature avocado trees, and the nature of the laurel wilt pathogen, the use of propiconazole has many limitations. Undoubtedly, the use of fungicides is a critical approach that must be implemented in an integrated management program of this disease; therefore, active ingredients with lower inhibition or fungicidal thresholds, longer half-life, and higher xylem mobility need to be explored. These active compounds should be formulated to allow root uptake and movement within the vascular system of large hardwood trees.

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