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Effect of Propiconazole on Laurel Wilt Disease Development in Redbay Trees and on the Pathogen In Vitro

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Abstract. Laurel wilt is a vascular disease of Lauraceous plants caused by a fungus (*Raffaelea* spp.) that is vectored by a recently introduced, nonnative ambrosia beetle (*Xyleborus glabratus*). The disease is devastating to redbay (*Persea borbonia*) trees in forests, parks, and residential landscapes in the southeastern United States, and management strategies for reducing its impact are needed. In this study, the systemic fungicide propiconazole completely inhibited mycelial growth of *Raffaelea* spp. in vitro at concentrations 0.1 parts per million (ppm) or greater and was fungitoxic at 1 ppm or greater, whereas the fungicide thiabendazole was less inhibitory. None of the ten mature redbay trees that received root-flare injections of propiconazole developed crown wilt symptoms for at least 30 weeks after being inoculated with *Raffaelea* spp., whereas nine of ten untreated control trees wilted in more than one-third of their crowns. Propiconazole was retained in the stem xylem for at least 7.5 months after injection but was more frequently detected in samples from trees injected 4.5 months earlier and was not well detected in small-diameter branches. Results suggest that propiconazole may be useful in preventing laurel wilt in redbay, but limitations and questions regarding duration of efficacy, rate of uptake, and efficacy under different levels of disease pressure remain.

Key Words. Ambrosia beetle; fungicide injection; laurel wilt; *Persea borbonia*, propiconazole, *Raffaelea*; redbay; thiabendazole; vascular disease; *Xyleborus glabratus*.

Laurel wilt is a vascular disease of redbay (*Persea borbonia*) and other plants in the family Lauraceae. It is caused by a fungus that is introduced into host plants by the redbay ambrosia beetle, *Xyleborus glabratus* (Fraedrich et al. 2008). The redbay ambrosia beetle is native to Asia but was first detected in North America in a survey trap at Port Wentworth, Georgia, U.S., in 2002 (Rabaglia et al. 2006). Since then, both the beetle and its associated fungus have been consistently recovered from dead and diseased redbays in areas of extensive redbay mortality in South Carolina, Georgia, and Florida. The fungus is a previously undescribed species of *Raffaelea*, an asexual stage of fungi in the genus *Ophiostoma*. Female redbay ambrosia beetles carry spores of *Raffaelea* spp. in mandibular mycangia and transmit them to host trees by boring into the sapwood of stems and branches. As the pathogen moves systemically, affected trees exhibit rapid wilting of foliage and a black discoloration in the xylem. In Florida, laurel wilt has caused mortality rates of greater than 90% within 2 years among redbay stems greater than 2.5 cm (1 in) in diameter (Fraedrich et al. 2008). The laurel wilt pathogen has also been recovered from diseased plants of other Lauraceous species in the southeastern United States, including sassafras (*Sassafras albidum*), pondberry (*Lindera melissifolia*), pondspice (*Litsea aestivalis*), and avocado (*Persea americana*) (Fraedrich et al. 2008; Mayfield et al., in press).

In addition to its potential ecologic impacts in natural forests and ecosystems, laurel wilt causes mortality of redbay shade trees in parks, residential neighborhoods, and other public landscapes (Mayfield 2007). Management tools are desired by homeowners and park managers to help protect landscape redbays and other hosts from laurel wilt and to minimize the associated aesthetic and economic impacts. Injection of systemic fungicides has been used to protect other tree species from similar vascular diseases. For example, injection of American elms (*Ulmus americana*) with the fungicides propiconazole and thiabendazole have been used to prevent mortality caused by Dutch elm disease (Haugen and Stennes 1999). Propiconazole injections have also been used to protect live oaks (*Quercus virginiana*) (Appel and Kurdyla 1992) and other species in the red and white oak groups (Osterbauer and French 1992; Ward et al. 2004; Eggers et al. 2005) from development of oak wilt.

The objectives of this study were 1) to determine the minimum effective concentrations (MECs) of propiconazole and thiabendazole to inhibit the laurel wilt pathogen (*Raffaelea* spp.) in vitro; 2) to evaluate the effect of root-flare injections of propiconazole on laurel wilt development in redbay trees; and 3) to evaluate the distribution and retention of propiconazole in stems and branches of injected redbays.

METHODS

Effect of Fungicides In Vitro

The effect of propiconazole (Alamo®; Syngenta Crop Protection, Inc., Greensboro, NC) and thiabendazole (Arbotect® 20-S; Syngenta Crop Protection, Inc.) on vegetative growth of the laurel wilt pathogen *Raffaelea* spp. was tested on fungicide-amended potato dextrose agar acidified with 50% lactic acid (APDA) in Petri plates (Difco Laboratories 1953). Fungicides were added to plates by mixing appropriate amounts of stock or diluted fungicide solution with the liquid APDA media before pouring plates. Treatments consisted of media amended with 50, 10, 1, 0.1, or 0.01 parts per million (ppm) of active ingredient for each fun-

gicide plus an unamended control. Six replicate plates were used per treatment. An isolate (FG) of the pathogen was obtained from the sapwood of diseased redbay at Ft. George Island, Florida, U.S. (isolate provided by S.W. Fraedrich, USDA For. Serv., Athens, GA) and the fungus was grown for 1 week on unamended APDA at room temperature. One 5 mm (0.2 in) agar plug with mycelium was removed from a Petri plate and placed top down onto each treatment plate. Two linear measurements (separated by a 45° angle) of mycelial growth were recorded on each plate at 7 and 14 days by measuring the distance between the edge of the inoculum plug and the edge of the colony. Inoculum plugs from plates with no mycelial growth after 14 days were transferred to unamended APDA for 1 week and examined to determine whether the fungus was dead (indicating fungitoxicity) or alive (indicating fungistatic activity only).

Mean cumulative linear mycelial growth, mean growth rate, and mean percent growth inhibition were calculated for each treatment. Percent growth inhibition was calculated as the difference between mean growth on control plates and growth on fungicide-amended plates, expressed as a percentage of the mean growth on control plates. One-way analysis of variance and Tukey's honestly significant difference test were used to evaluate differences between treatment means. Minimum effective concentrations for each fungicide were determined as the lowest experimental concentration at which mycelial growth was completely inhibited.

Inoculation Trial: Effect of Propiconazole Injections on Laurel Wilt Development in Redbay

The effect of propiconazole injections on the development of laurel wilt disease was evaluated at Ft. Clinch State Park in Fernandina Beach, Florida, U.S. (30.67473° N, 81.43475° W). The study site consisted of a maritime hardwood hammock forest dominated by live oak, redbay, laurel oak (*Quercus hemisphaerica*), southern magnolia (*Magnolia grandiflora*), and sabal palm (*Sabal palmetto*). Twenty mature redbay trees located along a hiking trail were selected for treatment. Trees ranged from 17 to 39 cm (6.8 to 15.6 in) diameter at breast height (dbh), had visibly healthy crowns, and were located at least 5.2 m (50 ft) apart. Tree diameters were sorted smallest to largest to create ten pairs of trees with similar diameter. One tree in each pair was randomly assigned to receive fungicide injection; the other tree served as an untreated control. Fungicide-treated trees received propiconazole (Alamo®) at the maximum label rate of 20 mL (0.7 fl oz) product diluted in 0.3 L (0.08 gal) of water for each 2.5 cm (1 in) of trunk diameter, equaling 1.2 g (0.04 oz) active ingredient per 1 cm (0.4 in) dbh. The amount of water used for dilution was reduced to 30% of the recommended volume (1 L, 0.26 gal) resulting from very slow uptake times experienced in pretrial practice applications. Injections were made using Rainbow Treecare Scientific Advancement's standard macroinfusion protocol (Rainbow Treecare Scientific Advancements 2005) (Figure 1A). Injections were made between 28 March and 5 April 2007.

On 19 April 2007 (2 to 3 weeks after fungicide injection), all 20 trees (fungicide-treated and control trees) were inoculated with *Raffaelea* spp. Two isolates of the fungus were used, one from Ft. George Island, Florida (FG) and one from Hilton Head Island, South Carolina (HH5) (isolates provided by S.W. Fraedrich, USDA For. Serv., Athens, GA). Isolates were selected based on either their origin from a local source of diseased redbays (FG) or successful use in other redbay inoculation experiments (HH5) (Fraedrich et al. 2008). Isolates were randomly assigned to each pair of trees with five pairs receiving the FG isolate and five receiving the HH5 isolate. Inoculum was produced by growing isolates for 1 week on unamended APDA at

Figure 1. Macroinfusion of redbay tree with the fungicide propiconazole (A). Inoculation of redbay trees by placing an agar plug with the laurel wilt pathogen (*Raffaelea* spp.) into a hole in the outer xylem (B).

room temperature. A drag plane was used to thin the outer bark of each tree at breast height, and a cork borer was used to make a 5 mm (0.2 in) diameter hole penetrating approximately 3 mm (0.1 in) into the outer sapwood. One mycelium-bearing, 5 mm (0.2 in) agar plug was cut from the source colony plates and placed top side inward into the hole in the sapwood (Figure 1B). Inoculum plugs were covered with moist paper towels and secured to the trunk with duct tape. Five additional untreated redbay trees received a sterile agar plug (no mycelium) in the same manner (Table 1).

Trees were evaluated for evidence of laurel wilt symptoms after 6 weeks and then every 3 to 10 weeks thereafter for 30 weeks. Each redbay crown was given a rating of 0 (healthy), 1 (wilt symptoms comprising up to one-third of the crown), or 2 (wilt symptoms comprising greater than one-third of the crown). At each rating period, trees with a crown rating of 2 were felled and wood samples taken from the stem and branches to determine the presence of *Raffaelea* spp. The first three trees to develop a crown rating of 2 were debarked along the entire main stem from the stump to a diameter of approximately 2.5 cm (1 in) and examined for evidence of black discoloration in the sapwood. Debarking of subsequent trees with crown ratings of 2 was done in spot-check fashion on the trunk and branches. Trees with crown ratings of 0 or 1 were left standing. Four sapwood plugs from standing trees were obtained with an increment hammer after thinning the outer bark with a drag plane at four locations approximately 1.8 m (6 ft) above the ground. To test for the presence of the *Raffaelea* fungus, wood samples approximately 0.5 cm^2 (0.08 in^2) showing characteristic xylem discoloration were surface-sterilized by soaking in 5% hypochlorite solution for 30 sec. After surface sterilization, the wood samples were plated on cycloheximide streptomycin malt agar (CSMA), a medium selective for *Ophiostoma* and their related anamorphs (Harrington 1981). The isolations were then incubated at room temperature and examined daily for the presence of fungal growth. Isolations were scored as positive when fungal cultures exhibiting the typical morphologic characteristics of *Raffaelea* spp. were observed.

At the termination of the experiment in mid-November 2007 (approximately 6 months after pathogen inoculation), final crown ratings were made and a χ^2 test for homogeneity was used to test the null hypothesis that the percentages of trees with a crown rating of 2 did not differ between the fungicide-treated group and the untreated control group. The null hypothesis was to be rejected if more than 20% of the fungicide-treated trees reached a crown rating of 2. The test was invalidated if fewer than 60% of the control trees reached a crown rating of 2.

Table 1. Number and diameter (dbh) of redbay trees receiving fungicide injection and/or inoculation treatments at Fort Clinch State Park, FL, U.S.

Tree injection treatment	Tree inoculation treatment[x]	No. of trees	dbh (cm)	
			Mean (SE)	Range
Propiconazole[z]	*Raffaelea* spp. plug	10	23.8 (1.7)	16.8–35.8
None	*Raffaelea* spp. plug	10	24.8 (2.0)	16.8–39.4
None	Sterile agar plug	5	15.1 (0.3)	14.2–16.0
Propiconazole[y]	None	7	23.4 (1.6)	16.5–29.2

[z]Trees injected 28 March to 5 April 2007.
[y]Two trees injected 30 March to 5 April 2007; five trees injected 27–28 June 2007. These trees were used and evaluated in the bioassay trial only.
[x]Trees inoculated 19 April 2007.

Bioassay: Retention of Propiconazole in Redbay Stems and Branches

In November 2007, a bioassay was conducted to determine if propiconazole was distributed to and retained in the stem and branches of fungicide-treated redbay trees. This included the ten trees that were part of the inoculation study described previously injected with propiconazole 7.5 months before the bioassay. An additional seven redbays that were not part of the inoculation trial but were injected with propiconazole either 7.5 months before (two trees) or 4.5 months before (five trees), were also included in the bioassay (Table 1). Wood samples from the five redbays that received no fungicide and sterile agar plugs in the inoculation experiment described previously were also included in the bioassay as controls (Table 1). In mid-November 2007, four sapwood plugs from each tree were obtained with an increment hammer after thinning the outer bark with a drag plane at four locations approximately 1.8 m (6 ft) above the ground. Live outer branches were clipped from six evenly distributed locations throughout the crown of each tree using a pole pruner. A twig section approximately 8 to 13 mm (0.32 to 0.52 in) in diameter and 12 cm (4.8 in) long was retained from each of the six branches per tree.

Bioassay plates were prepared using a spore suspension of the HH5 isolate of *Raffaelea* spp. The isolate was grown on APDA for approximately 7 days. Conidial suspensions were collected by flooding plates with a few milliliters of sterile water, loosening conidia with a glass rod, and passing the suspension through four layers of cheesecloth. The spore concentration of the suspension was estimated using a hemacytometer and adjusted to approximately 2.45×10^5 conidia/mL (7.25×10^6 conidia/oz). The spore suspension was evenly sprayed as a light mist onto CSMA plates. Twig sections were debarked, and two discs 3 to 5 mm (0.12 to 0.2 in) thick were cut from each of the six branches per tree and placed on the surface of spore-seeded bioassay plates (four disks per plate, three plates per tree). Also, the four sapwood plugs removed from the main stem of each tree were debarked and pressed into the agar of an additional spore-seeded plate. Plates were incubated at room temperature for 9 days.

After mycelia had grown on all surfaces of wood discs or plugs from untreated trees (9 days), the inhibition of growth on wood from fungicide-treated trees was scored using a modification of the system used by Stennes and French (1987). A score of 0 indicated mycelia growth over the entire disk or plug; 1 indicated inhibition of mycelial growth only on the top of the cross-section of the disc (or only on a portion of the plug); 2 indicated inhibition of mycelial growth on the entire surface of the disc or plug; and 3 indicated complete inhibition of growth on the disc or plug plus a zone of inhibition in the surrounding agar. The percentage of branches exhibiting inhibition of the fungus was calculated as 100 × the number of branches with discs scoring 1 or greater divided by the number of discs per tree. The percentages of stem plugs exhibiting inhibition (score 1 or greater) and inhibition zones in the surrounding agar (score 3) were also calculated per tree. A two-sample t-test was used to compare the mean percentage of samples exhibiting growth inhibition between trees injected 4.5 months earlier and trees injected 7.5 months earlier.

Table 2. Mean linear mycelial growth, growth rate, and percent growth inhibition of *Raffaelea* spp. on fungicide-amended and unamended (control) acidified potato dextrose agar (APDA) in vitro.[z]

Fungicide	Fungicide concentration (ppm)	Accumulated linear growth (mm) 1 week	Accumulated linear growth (mm) 2 weeks	Linear growth rate (mm/day)	Percent growth inhibition	Status of inoculum after 2 weeks
Propiconazole	0.01	4.1 c	10.8 c	0.8 c	84.0 b	Alive
	0.1	0.0 d	0.0 d	0.0 d	100.0 a	Alive
	1	0.0 d	0.0 d	0.0 d	100.0 a	Dead
	10	0.0 d	0.0 d	0.0 d	100.0 a	Dead
	50	0.0 d	0.0 d	0.0 d	100.0 a	Dead
Thiabendazole	0.01	29.2 a	66.1 ab	4.7 ab	2.3 d	Alive
	0.1	25.8 b	64.2 b	4.6 b	5.2 c	Alive
	1	4.7 c	10.3 c	0.7 c	84.9 b	Alive
	10	0.0 d	0.0 d	0.0 d	100.0 a	Alive
	50	0.0 d	0.0 d	0.0 d	100.0 a	Alive
Control	0	27.9 a	67.7 a	4.8 a	N/A	Alive

[z]Within columns, means followed by the same letter are not significantly different ($P > 0.05$) as determined by one-way analysis of variance and Tukey's honestly significant difference test.
N/A = not applicable.

RESULTS

Effect of Fungicides In Vitro

Results of the in vitro fungicide evaluation are summarized in Table 2. Propiconazole completely inhibited mycelial growth of *Raffaelea* spp. at experimental concentrations at or above 0.1 ppm (the MEC) and was fungitoxic at or above 1 ppm (Figure 2). Pathogen growth on propiconazole-amended plates was 84% inhibited at the lowest concentration tested (0.01 ppm). In contrast, the MEC for thiabendazole was 10 ppm, and this fungicide was not fungitoxic at any concentration tested (up to 50 ppm). Mean linear growth and growth rates of *Raffaelea* spp. on plates amended with 0.01 ppm thiabendazole were not significantly different from control plates. The pathogen on unamended APDA grew an average of 5 mm/day over a 14-day period.

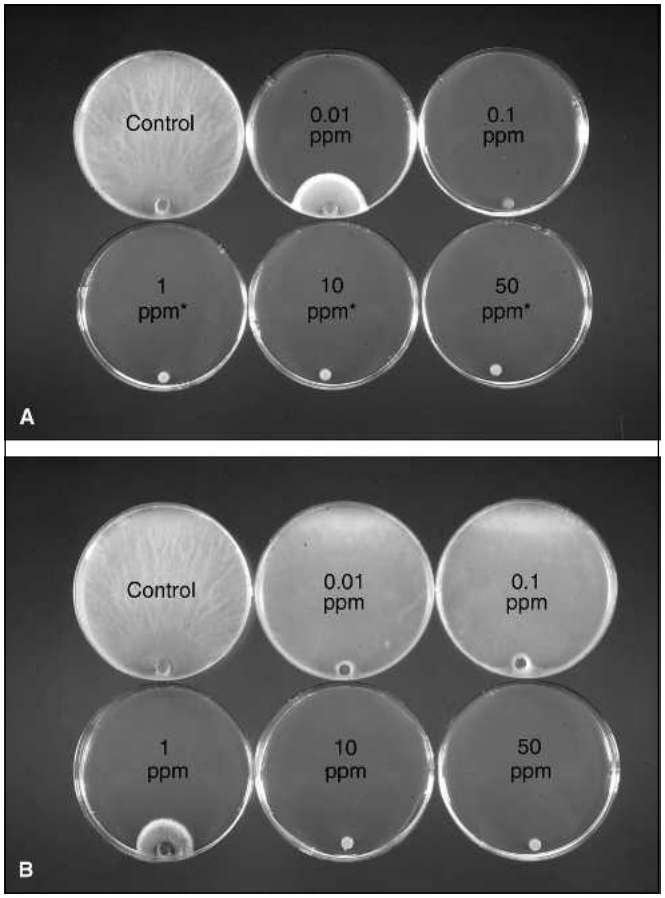

Figure 2. Mycelial growth of the laurel wilt fungus *Raffaelea* spp. on acidified potato dextrose agar (APDA) amended with the fungicides propiconazole (A) and thiabendazole (B) after 19 days. Fungicide concentrations are indicated in parts per million (ppm) on each plate with asterisks indicating fungitoxic levels.

Inoculation Trial: Effect of Propiconazole Injections on Laurel Wilt Development in Redbay

The ten redbay trees that were injected with propiconazole and subsequently inoculated with *Raffaelea* spp. retained healthy crowns (rating = 0) through the duration of the study (30 weeks postinoculation). *Raffaelea* spp. was not reisolated from the sapwood of these trees. In contrast, nine of the ten redbays that were inoculated with *Raffaelea* spp. but not injected with fungicide developed wilt. Seven of the trees had wilt symptoms in more than one-third of the crown after 9 weeks, and nine had symptoms to this extent by 12.5 weeks (Table 3). Felling and debarking of wilted trees revealed continuous streaks of black discoloration in the sapwood that spiraled around the stem and extended into the branches (Figure 3). *Raffaelea* spp. was successfully reisolated from the discolored sapwood in eight of the nine wilted trees (Table 3). No wilt symptoms were observed in the five redbays that received only sterile agar plugs nor was the pathogen recovered from sapwood samples (Table 3).

Bioassay: Retention of Propiconazole in Redbay Stems and Branches

Stem sapwood plugs from all but one of the redbay trees that were injected with propiconazole inhibited the growth of *Raffaelea* spp. in the bioassay, indicating that fungicide was retained in the vascular system for at least the first 4.5 to 7.5 months postinjection (Table 4). No inhibition of *Raffaelea* spp. growth was observed on samples from control (untreated) trees. On average, among trees with samples exhibiting inhibition, 84% to 95% of the stem plugs per tree showed evidence of fungicide

Table 3. Percentage of redbay trees with greater than one-third crown wilt symptoms and from which *Raffaelea* spp. was reisolated after fungicide injection and pathogen inoculation treatments.

Tree injection treatment	Tree inoculation treatment	No. of trees	Cumulative number of trees with wilt symptoms in more than one-third of the crown by time since inoculation				Number of trees from which *Raffaelea* spp. was reisolated[y]
			6 weeks	9 weeks[z]	12.5 weeks[z]	30 weeks[z]	
Propiconazole	*Raffaelea* spp. plug	10	0	0	0	0	0
None	*Raffaelea* spp. plug	10	3	7	9	9	8
None	Sterile agar plug	5	0	0	0	0	0

[z]Among trees inoculated with *Raffaelea* spp., the proportion of trees with greater than one-third crown wilt symptoms significantly differed between the propiconazole group and the untreated group after 9 weeks ($\chi^2 = 10.77$, $P = 0.001$) and 12.5 or more weeks ($\chi^2 = 16.36$, $P = 0.0001$).
[y]Among trees inoculated with *Raffaelea* spp., the proportion of trees from which *Raffaelea* spp. was reisolated significantly differed between the propiconazole group and the untreated group ($\chi^2 = 13.33$, $P = 0.0003$).

Figure 3. Black discoloration in the outer sapwood of a redbay tree 6 weeks after artificial inoculation with the laurel wilt pathogen, *Raffaelea* spp.

retention (score = 1 or greater). Trees injected 4.5 months earlier had a significantly higher mean percentage of stem plugs exhibiting inhibition zones in the agar than trees injected 7.5 months earlier (Table 4).

Only five of the 12 trees that were injected with propiconazole 7.5 months earlier exhibited evidence of fungicide retention in the outer branches (Table 4). Of these trees, only approximately 33% of the branch samples per tree exhibited evidence of fungicide retention. Four of the five trees that were injected 4.5 months earlier exhibited evidence of fungicide in the branches, but the mean percent fungicide distribution in the crowns of these trees was only 29%. None of the branch sample discs produced a zone of growth inhibition in the surrounding agar (Table 4).

DISCUSSION

The laurel wilt pathogen, *Raffaelea* spp., was more sensitive to propiconazole than to thiabendazole in vitro. Propiconazole was fungitoxic at 1 ppm and completely fungistatic at 0.1 ppm. This MEC of 0.1 ppm is comparable to that reported in sensitivity tests of the oak wilt fungus (*Ceratocystis fagacearum*) to propiconazole on fungicide-amended PDA (Appel and Kurdyla 1992; Wilson and Forse 1997). As a result of its greater efficacy in vitro as well as the lower volume of water required to dilute the product for tree injection (allowing shorter application times), this study focused on propiconazole in the tree injection trial rather than thiabendazole. Nonetheless, although it was not fungitoxic at the concentrations tested in this study, thiabendazole did completely inhibit growth of *Raffaelea* spp. at concentrations down to 10 ppm and may be worthy of further evaluation in tree injection trials that test for efficacy to prevent laurel wilt disease.

Root-flare injections with propiconazole may represent a much-needed management option for preventing laurel wilt disease development in mature redbays. The results of this study clearly demonstrate that propiconazole, injected in advance of inoculation with the laurel wilt pathogen, can inhibit the spread of this pathogen in xylem tissue and prevent laurel wilt. Several unanswered questions remain, however, about the efficacy of propiconazole injections for prevention of laurel wilt. Although the trees in this study will continue to be monitored as the incidence of laurel wilt in the area increases, this study did not evaluate the maximum length of time that trees can be protected. Osterbauer and French (1992) found that propiconazole was retained in the vascular system of root-flare-injected oaks (*Quercus rubra* and *Q. ellipsoidalis*) in Minnesota up to 1 year after treatment, but not after 20 months, and suggested that trees should be retreated approximately every 2 years for prevention of oak wilt. In this study, although nearly all (16 of 17) treated trees exhibited evidence of fungicide retention in the bioassay, the percentage of stem sapwood samples per tree exhibiting fungicide retention was significantly greater in trees treated 4.5 months previously than in trees treated 7.5 months previously (Table 4), suggesting that the fungicide may have already begun to degrade within the first 8 months posttreatment. The ability of the fungicide to protect trees inoculated later than 3 weeks postinjection or at multiple locations on a stem (as may happen with attacks by the redbay ambrosia beetle) was not evaluated in this study.

Table 4. Results of bioassay testing for fungicide retention (indicated by inhibition of *Raffaelea* spp. growth) in redbay stem plugs and branch discs plated on spore-seeded, cycloheximide streptomycin malt agar (CSMA).

Sample	Tree injection treatment	Months since fungicide injection	No. of trees	No. of trees with samples exhibiting inhibition	Percentage of samples per tree exhibiting inhibition[z]		Percentage of samples per tree exhibiting inhibition zone in the agar[z]	
					Mean	Range	Mean	Range
Stem plugs	Propiconazole	7.5	12	11	84 a	25–100	27 a	0–75
	Propiconazole	4.5	5	5	95 a	75–100	80 b	50–100
	None	N/A	5	0	N/A	N/A	N/A	N/A
Branch discs	Propiconazole	7.5	12	5	33 a	17–83	0	0
	Propiconazole	4.5	5	4	29 a	17–50	0	0
	None	N/A	5	0	N/A	N/A	N/A	N/A

[z]Mean and range are based only on data from trees in which inhibition of mycelial growth was exhibited. Within columns, means followed by the same letter are not significantly different ($P > 0.05$) as determined by a two-sample t-test. Four stem plugs and six branch samples were collected per tree.
N/A = not applicable.

Uptake of fungicide solution by redbay trees in this study was relatively slow compared with the authors' experience with preventive root-flare injections on ring-porous species such as elms and oaks. An accurate uptake record was maintained for 12 redbay trees and indicated that the uptake process lasted an average of 53 min/L (0.26 gal) of solution, or approximately 156 min per tree (range, 30 to 360 min). The pronounced drought conditions at the time of injection may have contributed to the slow rate of uptake. At the start of injections in late March, the study site had received only 0.3 cm (0.12 in) of rain in the preceding 25 days, and the average monthly Palmer Drought Severity Index in northern Florida from July 2006 to June 2007 remained continuously negative and averaged –3.78 through the year leading up to the completion of the injections (National Climatic Data Center 2008). Thus, uptake times on redbay may have been better under conditions of more normal rainfall. The diffuse–porous nature of redbay xylem and the twisting growth habit of many redbay trunks may also have contributed to the slow uptake times relative to ring-porous and typically straighter-trunked tree species such as elms and oaks. The relatively slow rate of uptake may have also limited the distribution of propiconazole into the small branches of the crown, which exhibited rather limited fungicide retention.

The macroinfusion technique used in this study delivers a relatively large volume of pressurized fungicide solution into the xylem through holes drilled in the root flare. Macroinfusion provides more adequate and even distribution of fungicide into the canopy of treated trees than do alternative delivery methods such as microinjection (Haugen and Stennes 1999). In general, injection methods result in bark and sapwood injury when drilling the injection holes; such wounds can limit the number of times injections can be repeated and may cause sapwood discoloration or decay (Haugen and Stennes 1999). The authors are unaware, however, of less injurious delivery methods that would be as effective for treating wilt disease in mature trees.

Propiconazole has been used successfully in therapeutic (i.e., curative) treatments for oak wilt in red oaks exhibiting less than 25% crown wilt symptoms (Ward et al. 2005) and in combination with pruning in white oaks with 5% to 45% crown wilt symptoms (Eggers et al. 2005). Although this study did not evaluate therapeutic treatments, we suspect that such treatments on redbays would have to be implemented very early during laurel wilt symptom development as a result of the extremely rapid nature of disease progression once trees begin showing signs of wilt. Because wounding increases the attractiveness of the redbay trees to the redbay ambrosia beetle (Hanula et al., in press), the use of pruning in combination with injection treatments is probably not an effective option for treating laurel wilt.

As a result of the devastating effects of laurel wilt on populations of mature redbays and the rapid rate of spread of this disease geographically (estimated to be at least 32 km [19.2 mi] per year), we consider it important to report these results now rather than to wait for multiple years of efficacy data. In this study, propiconazole was toxic to the pathogen at low concentrations in vitro, prevented development of wilt symptoms in inoculated redbays, and was retained in the sapwood (primarily in the trunk) for at least 7.5 months after injection. Propiconazole injections could be potentially useful to protect large, high-value redbay shade trees in parks, residential neighborhood yards, and historic sites that are currently succumbing to this disease and for which no other preventive management options currently exist. Although the results in this study demonstrate that the systemic fungicide propiconazole could be useful for the protection of redbay trees from laurel wilt, additional research is clearly needed to evaluate delivery methods, rate and season of application, and the effect of various levels of inoculation pressure (i.e., number and frequency of inoculation events or beetle attacks) on the long-term efficacy of propiconazole and other fungicides.

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Résumé. La flétrissure du laurier est une maladie vasculaire qui affecte les plants de la famille des Lauracées et qui est causée par un champignon (*Raffaelea* spp.) qui est véhiculée par un scolyte non indigène récemment introduit, le *Xyleborus glabratus*. Cette maladie est dévastatrice pour les lauriers rouges (*Persea borbonia*) dans les forêts, les parcs et les aménagements paysagers résidentiels du Sud-est des États-Unis et des stratégies de egstion pour réduire son impact sont requises. Dans cette étude, le fongicide systémique propiconazole a entièrement inhibé la croissance in vitro du mycélium de *Raffaelea* spp. à des concentrations de 0,1 partie par million (ppm) ou plus fortes et il s'avérait phytotoxique à des concentrations d'au moins 1 ppm tandis que le fongicide thiabendazole inhibait moins la maladie. Aucun des 10 lauriers rouges qui ont reçus des injections basales de propiconazole ont développé des symptômes de flétrissure de la cime pour au moins 30 jours après la première inoculation avec *Raffaelea* spp. alors que neuf des dix arbres du groupe témoin ont subi une flétrissure sur au moins le tiers de leur cime. Le propiconazole était retenu dans le xylème de la tige durant au moins 7,5 mois après l'injection, mais il était moins fréquemment détecté dans les échantillons d'arbres qui avaient été injectés 4,5 mois plus tôt et il n'était pas facilement détecté dans les branches de faibles diamètres. Les résultats suggèrent que le propiconazole pourrait être très utile pour prévenir la flétrissure chez le laurier rouge, mais que des limitations et des questionnements subsistent par rapport à la durée de l'efficacité, le taux d'assimilation et l'efficacité sous différentes pressions par la maladie.

Zusammenfassung. Die Lorbeerwelke ist eine vaskuläre Erkrankung von Lauraceen, verursacht durch einen Pilz (Raffaelea spp.), welcher durch einen kürzlich eingeführten, nonnativen Ambrosiuskäfer (*Xyleborus glabratus*) verbreitet wird.Im Südosten der USA dezimiert diese Krankheit die Rotlobeerbäume (Persea borbonia) in Wäldern, Parks und Privatgärten, was eine Bekämpfungsstrategie zur Eindämmung dieser Krankheit erforderlich macht. In dieser in-vitro-Studie hemmte das systemische Fungizid Propikonazol in der Konzentration von 0,1 ppm oder

größer das Wachstum von Myzel, war tötlich bei 0.1 ppm oder größer, während das FungizidThiabendazol schlechter abschnitt. Keiner der zehn Rotlobeerbäume, die Propikonazol über die Wurzel appliziert bekamen, entwickelte für mindestens 30 Wochen nach der Okkulation von Raffealea spp. Krankheitssymptome, während neun von zehn Kontrollbäumen in mehr als einem Drittel ihrer Krone Welke zeigten. Propikonazol konnte bis zu 7,5 Monate nach der Injektion im Xylem nachgewiesen werden, aber es war häufiger nachgewiesen in Bäumen, die 4,5 Monate früher injiziert wurden sowie konnte in kleinen Ästen schlecht nachgewiesen werden. Die Ergebnisse führen zu der Annahme, dass Propikonazol bei der Bekämpfung von Lorbeerwelke nützlich sein könnte, aber die Begrenzungen und aufkommenden Fragen bezüglich der Dauer des Schutzes, der Aufnahmerate und der Effizienz unter verschiedenen Graden von Krankheitsbefall.

Resumen. El marchitamiento del laurel es una enfermedad de plantas Lauraceae causada por un hongo (*Raffaelea* spp.) cuyo vector es un escarabajo ambrosia no nativo recientemente introducido (*Xyleborus glabratus*). La enfermedad está desbastando a los árboles de *Persea borbonia* en bosques, parques, y áreas residenciales en el sudeste de los Estados Unidos, y se requieren estrategias de manejo para reducir su impacto. En este estudio, el fungicida sistémico propiconazole inhibió completamente el crecimiento micelial de *Raffaelea* spp. in vitro a concentraciones de 0.1 partes por millón (ppm) o mayores y fue fungitóxico a 1 ppm o mayores, mientras que el fungicida thiabendazole fue menos inhibitorio. Ninguno de los diez árboles maduros de Persea, que recibió inyecciones en la corona de la raíz de propiconazole, desarrolló síntomas de marchitamiento de la copa al menos por 30 semanas después de inoculados con *Raffaelea* spp. Mientras que nueve de los diez árboles no tratados de control se marchitaron en más de la tercera parte de sus copas. El propiconazole fue retenido en el xilema del tallo por al menos 7.5 meses después de las inyecciones, pero no fue detectado con frecuencia en ramas de diámetro pequeño. Los resultados sugieren que propiconazole puede ser útil para la prevención del marchitamiento del laurel en árboles de Persea, pero con limitaciones e inquietudes relacionadas con la duración de su eficacia, tasa de absorción, y eficiencia bajo diferentes niveles de presión remanente de la enfermedad.