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LAUREL WILT: A DANGEROUS NEW DISEASE OF AVOCADO IN THE WESTERN HEMISPHERE

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ABSTRACT: Laurel wilt kills American members of the Lauraceae plant family, including avocado (*Persea americana*). The disease is caused by a recently described fungus, *Raffaelea lauricola*, and is vectored by an ambrosia beetle from Asia, *Xyleborus glabratus*. Responses to laurel wilt were determined for 22 cultivars of avocado that are grown in Florida. Those with a West Indian pedigree (they are most important in Florida and the Dominican Republic) were most susceptible. *In vitro* and *in planta* studies were conducted to identify effective fungicides. Several chemistries impacted the fungus *in vitro*, but only demethylation inhibitors and thiabendazole provided significant disease control in greenhouse trials. Field investigations are underway to achieve high xylem concentrations of an effective triazole, propiconazole. Although it will be difficult to control this disease by managing *X. glabratus*, insecticides and repellents are also being examined, as are attractants for attract and kill strategies.

Avocado responds to infection by *R. lauricola* by accumulating phenolic substances and producing tyloses in vessel elements, typical host defense responses. Diagnostic PCR primers for the small subunit (SSU) ribosomal DNA of *R. lauricola* were developed and used with traditional and realtime PCR; they have enabled the sensitive detection and localization of *R. lauricola* in artificially inoculated plants, and have been valuable tools in studies on host:pathogen interactions, fungicidal control, epidemiology, and resistance. A species-specific diagnostic tool, now under development, will play an important role in laurel wilt interdiction and laurel wilt management in avocado production areas via sanitation. Early detection of the pathogen and disease are needed for quarantine, eradication, and sanitation efforts.

Key Words: *Raffaelea lauricola, Xyleborus glabratus*, redbay ambrosia beetle, avocado, *Persea americana*, quarantine, detection, sanitation, eradication

INTRODUCTION

In May 2002, an exotic ambrosia beetle, *Xyleborus glabratus*, was trapped at Port Wentworth, a maritime port outside Savannah, Georgia, USA (Rabaglia et al. 2006). A native of Asia (Bangladesh, Burma, India, Japan, and Taiwan), the insect had not been reported previously in the Western Hemisphere. Little concern was attached to its appearance until it was associated with a new disease. Laurel wilt affected a native component of forests in the southeastern United States, redbay (*Persea borbonia* Lauraceae), and was shown to be vectored by *X. glabratus* and caused by a previously unknown fungus, *Raffaelea lauricola* (Fraedrich et al. 2008; Harrington et al. 2008; Mayfield et al. 2008b).

Ambrosia beetles typically infest dead or stressed trees in which they establish gardens of ambrosia fungi (Harrington 2005). These fungi are coevolved symbionts of the beetles and are usually saprobes. They are carried in specialized structures in the insects (mycangia) and are the insect's sole or primary food source (the beetles do not consume wood). Laurel wilt is unusual, in that *X. glabratus* attacks healthy trees and its fungal symbiont, *R. lauricola*, is a virulent pathogen. Although the tight association between ambrosia beetles and their fungal symbionts suggests that *R. lauricola* entered the United States with *X. glabratus*, laurel wilt has not been reported in the beetle's Asian homeland.

In less than a decade, laurel wilt has spread throughout much of the southeastern Atlantic coastal plain (Figure 1). It now affects several other species in the Lauraceae, including a most important agricultural suscept, avocado (*Persea americana*) (Fraedrich et al. 2008; Mayfield et al 2008c). This rapid movement has resulted due to its efficient insect vector and the anthropogenic dissemination of infested materials. For example, a 100-km jump occurred after a hobbyist transported laurel wilt-affected wood from Jacksonville (Duval County) to Volusia County, Florida, and a 550-km jump to Mississippi probably involved similar activity (Chemically Speaking 2009; Hughes (unpubl.); Riggins et al. 2010).

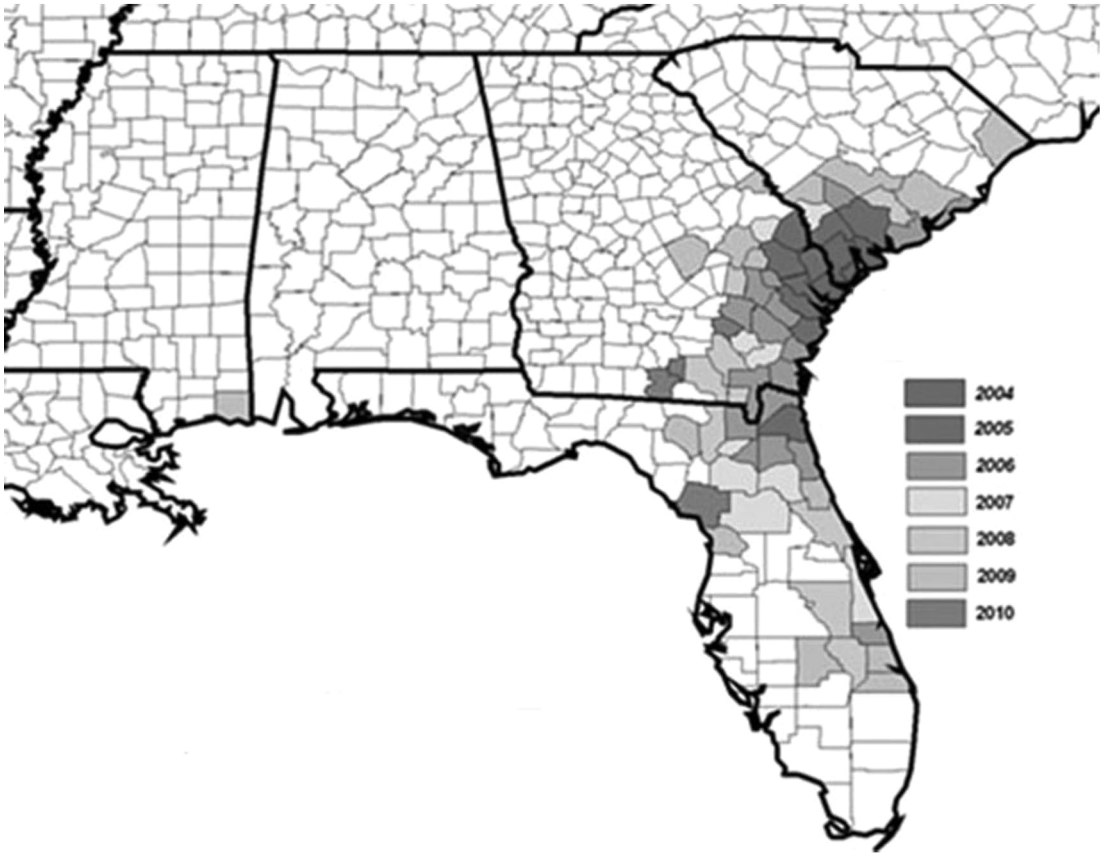

Figure 1. Distribution of laurel wilt (USDA, 2010).

Much remains to be learned about the relative susceptibilities of native and non-native hosts in the southeastern United States to laurel wilt and their attractiveness to *X. glabratus*. The abundance and distribution of these taxa probably plays an important role in the epidemiology of this disease (Koch and Smith 2009). Understanding these relationships will be important as the disease moves in the United States and if it is found in other avocado-producing countries.

Laurel wilt is an immediate threat to commercial avocado production in Florida, centered in Miami-Dade County, as well as the National Germplasm Repository for avocado in Miami (USDA/ARS). Elsewhere, major production throughout the Western Hemisphere, which includes seven of the world's top ten producers (Table 1), is at risk.

In 2006, avocado seedlings (unspecified cultivar) succumbed to artificial inoculation with *R. lauricola* in an incubator trial (Fraedrich et al. 2008), and in 2007, the first naturally affected tree (unknown cultivar) was reported in the Jacksonville area (Mayfield et al. 2008c). Residential avocado trees have continued to die as the disease moved south in the state but, as of June 2010, Florida's commercial avocado-production area had not been affected.

Table 1. Avocado production statistics, 2008[a]

Country	Area underProduction (hectares)	Total Production (metric tonnes)
Mexico	114,471	1,124,565
Chile	39,842	250,000
Indonesia	19,786	225,180
Dominican Republic	6,300	187,398
Colombia	18,470	183,968
Brazil	10,550	166,000
Peru	13,603	121,720
Spain	15,070	120,000
United States	29,473	114,305
South Africa	17,000	99,650
Global totals	423,624	3,532,011

[a]Figures from FAOSTAT (2010)

About 3.5 million metric tons (MMT) of avocado were harvested worldwide in 2008 (Table 1). Mexico was the most important producer, with the Dominican Republic and the United States, respectively, fourth and ninth globally. California and Florida are the primary producing states in the United States (USDA).

Three botanical races of avocado are recognized (Knight 2002; Scora et al. 2002). The Mexican (M) (*P. americana* var. *drymifolia*) and Guatemalan (G) (*P. americana* var. *guatemalensis*) races originated in the respective highlands of those countries, whereas the West Indian (WI) or lowland race (*P. americana* var. *americana*) arose on the Pacific coast of Central America. Due to their respective environmental adaptations, historical dissemination, and local market preferences, different cultivars are grown in different areas. For example, M, G, and MxG hybrid cultivars predominate in Mexico and California, but WI, G, and WIxG cultivars are most important in Florida and the Dominican Republic (Crane et al. 2007; Knight 2002). One MxG cultivar, Haas, accounts for 95% of all production in California, but 23 major and 20 minor cultivars are produced in Florida. Commercial production in both states relies on clonal scions that are grafted on clonal or seedling (most common) rootstocks, as does production that enters international trade.

We summarize recent work to understand how avocado responds to laurel wilt, and how the disease might be managed in the future.

MATERIALS AND METHODS

All experiments were conducted with grafted avocado plants that are used in commercial production (clonal scions on seedling rootstocks) (Ploetz et al. 2010; Ploetz and Perez 2010). Experiments were conducted under greenhouse or field conditions, depending on where in Florida laurel wilt had been documented during a given year, and the restrictions that were imposed by the Florida Department of Agriculture and Consumer Services (FDACS) on where field experiments with the invasive pathogen could be conducted. Greenhouse experiments were conducted in 2007 at quarantine facilities of FDACS, Division of Plant Industry, in Gainesville, FL, and in 2009 and 2010, under FDACS permits in a secure greenhouse at the University of Florida's Tropical Research and Education Center in Homestead. Field experiments were conducted in 2008 and 2009 at the University of Florida's Plant Science Research and Education Unit in Citra.

To induce disease, plants were artificially inoculated with isolates of *R. lauricola*. Either patches of mycelium were inserted in clefts cut 5 cm above the graft union or 100 ml of conidial suspensions (10^5 conidia ml^{-1}) were inserted in holes that were drilled in stems; all inoculation sites were wrapped in Parafilm.

Every 2–3 weeks after inoculation (WAI), field experiments were rated for external disease development on a 1–5 or 1–10 subjective severity scale (1 = no symptoms, and 5 or 10 = dead). In some cases, plants in the field were inoculated a second time several months after the first inoculation. Since cold temperatures killed all plants at the Citra field site during the winters of 2008 and 2009, data were not taken from those experiments the following year.

Greenhouse experiments were usually terminated 5 WAI. Beginning 2 WAI, weekly disease responses were recorded externally on the above scales; 5 WAI, plants were dissected to also record internal symptom severity (same scales) and the linear extent of symptom development (vascular discoloration).

Representative plants in all experiments were assayed for the causal fungus on a semi-selective medium (CSMA) that was developed by Harrington (1981). Host tissue from specific locations along the inoculated stems were assayed on the medium and via qPCR to determine the extent of colonization by *R. lauricola* and the relationship between colonization and symptom development.

Cultivar Experiments

In field experiments at Citra in 2008 and 2009, responses to laurel wilt were determined for 22 cultivars of avocado that are grown in Florida (Table 2). Cultivars were replicated six times in a randomized complete block design, and plants were inoculated and rated for disease three (2008) or two (2009) times.

Plant Size

Since large redbay plants developed laurel wilt symptoms more quickly and severely (Fraedrich et al. 2008), we investigated the influence of plant size on disease development on avocado. 'Simmonds' plants (susceptible WI cultivar) of different sizes were tested in an initial greenhouse experiment in 2007 (1-gallon and 7-gallon pots, unrecorded stem diameters), and a field experiment in Citra, Florida in 2008 with plants in 15-gallon, 7-gallon, and 3-gallon pots.

Stem diameters of the 3-gallon pot plants were recorded and used to assess the relationship between size and the severity of disease that developed.

Table 2. Laurel wilt response of avocado cultivars in field experiments

Cultivars	Genome[b]	2008	2009	2008-2009 Mean	Genome Mean[d]
Ettinger	GxM	n/t	2.9	2.9	
Hass	G(58%)xM(42%)	3.9	2.9	3.4	
Winter Mexican	GxM	n/t	1.8	1.8	2.7 b
Bacon	G(94%)	n/t	2.2	2.2	
Reed	G(100%)	n/t	3.5	3.5	2.8 b
Brogdon	GxMxWI	4.1	4.1	4.1	4.1 ab
Beta	GxWI	n/t	3.2	3.2	
Choquette	GxWI	3.5	3.6	3.6	
Hall	GxWI	3.3	4.9	4.1	
Lula	GxWI	5.5	3.1	4.3	
Miguel	GxWI	6.4	3.7	5.1	
Monroe	GxWI	5.2	2.9	4.1	
Tonnage	GxWI	n/t	3.5	3.5	4.0 ab
Bernecker	WI	5.2	4.2	4.7	
Catalina	WI	5.1	5.1	5.1	
Day	WI	4.4	n/t	4.4	
Donnie	WI	6.3	4.5	5.4	
Pollack	WI	n/t	3.7	3.7	
Russell	WI	n/t	5.6	5.6	
Simmonds	WI	6.3	5.8	6.1	
Trapp	WI	n/t	3.3	3.3	
Waldin	WI	n/t	4.3	4.3	4.7 a

[a] Plants were artificially inoculated with mycelium (2008) or conidia (2009) of *Raffaelea lauricola* in field experiments at University of Florida's Plant Science Research and Education Unit in Citra. Data are mean disease severities for three (2008) or two (2009) experiments on a given cultivar. Experiments in 2008 were originally rated on a 1-5 scale, and those in 2009 on a 1-10 scale (1=no symptoms and 5 or 10=dead). To facilitate direct comparisons with data from 2009, the 2008 scores were doubled.

[b] Based on SSR data from Schnell et al. (2003), genome indicates whether a cultivar has a pure Guatemalan (G) (*Persea americana* var. *guatemalensis*) or West Indian (WI) (*P. americana* var. *americana*) background, or whether it is a GxWI hybrid, G x Mexican (M) (*P. americana* var. *drymifolia*) hybrid, or a complex GxMxWI hybrid. Percentage figures for some of the cultivars indicate relative proportions of the various racial backgrounds based on DNA sequence data from (Chen et al. 2009). Previous publications indicated different pedigrees for Bacon (M or GxM), Brogdon (unknown), and Hass (G or complex). Note the prevalence of GxWI and WI cultivars, which predominate in commercial production in Florida.

[c] n/t=not tested.

[d] Means for genomes are separated with DMRT, $P<0.05$.

Since large plants are expensive and unavailable for many cultivars, we were also interested in determining whether small plants would develop reliable symptoms if they were inoculated multiple times. To investigate this possibility, newly grafted plants (≤ 1 cm diameter) of 'Choquette' GxWI, 'Donnie' WI, 'Haas' GxM, 'Lula' GxWI, 'Monroe' GxWI, and 'Simmonds' were inoculated either 1 or 5 times.

Host:Pathogen Interactions

Greenhouse studies were conducted in Homestead, Florida to determine the relationship between internal and external symptom development on 'Simmonds' and colonization of the host by *R. lauricola*. Symptom development was rated as above, and presence of the pathogen was assessed with CSMA and qPCR. The interaction between avocado and *R. lauricola* was also studied by examining inoculated tissue via light and scanning electron microscopy.

Fungicide Experiments

Twenty-eight different fungicides in 18 chemical groups were tested for inhibiting the growth of *R. lauricola in vitro* (FRAC 2010; Ploetz and Perez 2010). The best products from these assays were tested further against the disease in three greenhouse trials on 'Simmonds'. Plants were treated with fungicides in one of three ways: 1) drench applications in which 1 L suspensions of fungicides were poured on the soil surface; 2) bark-directed applications in which 100 ml of fungicide suspensions were sprayed on trunk and branch surfaces in 2% Pentrabark (AGRICHEM, 5171 Morning Song Dr., Medina OH 44256); and 3) granular applications (only Prophesy, a granular formulation of propiconazole, in Experiment 1). After three weeks, plants were inoculated with *R. lauricola*. Mock-inoculated plants were treated with water.

After five weeks, plants were evaluated externally and internally for disease incidence and severity (1–10 subjective scale, wherein 1 = no symptoms/healthy; and 10 = dead). Above and below the inoculation site, the linear extent and severity of internal vascular discoloration (1–10 scale) was recorded after bark was removed from the stem surface.

Diagnosis

Diagnostic PCR primers for the small subunit (SSU) ribosomal DNA of *R. lauricola* were developed and used with traditional and realtime PCR (Dreaden et al. 2008).

RESULTS AND DISCUSSION

There were significant differences in the severity of disease that developed on different cvs, and cvs with a WI background were generally more susceptible than those with other pedigrees (Table 2). Future work will examine resistance in a greater diversity of M and G germplasm, as the few cultivars that were tested with these pedigrees developed less severe disease. Ultimately, disease tolerant germplasm will either be selected or developed in screening trials.

'Simmonds,' a WI cv, which comprises ca 35% of commercial production in South Florida, has been used as a standard suscept in different studies. Disease severity increased significantly on 'Simmonds' as plant size (stem diameter) increased (Figure 2). Newly grafted plants (≤ 1 cm dia) of 'Choquette' GxWI, 'Donnie' WI, 'Haas' GxM, 'Lula' GxWI, 'Monroe' GxWI, and 'Simmonds' developed little disease and recovered within 2 months of inoculation, regardless of genotype (data not shown). The apparent requirement for large plants in disease studies

complicates the identification of resistant genotypes of avocado, as these plants are expensive and unavailable for many cultivars.

Internal and external disease development on 'Simmonds' were correlated (Figure 3). A threshold for xylem dysfunction (internal symptoms) was noted; external symptoms (e.g., wilting and defoliation) developed only after relatively severe internal symptoms developed. Latent infection was uncommon, in that *R. lauricola* was isolated on CSMA only from discolored xylem of inoculated 'Simmonds', and was detected infrequently in the advance of such symptoms with qPCR. Although the pathogen colonizes host xylem, basipetal movement of 2 cm was common within four weeks of inoculation; thus, movement of this pathogen among trees via root grafts may be possible. Greater understandings are needed of the movement of *R. lauricola* in naturally and artificially infected trees.

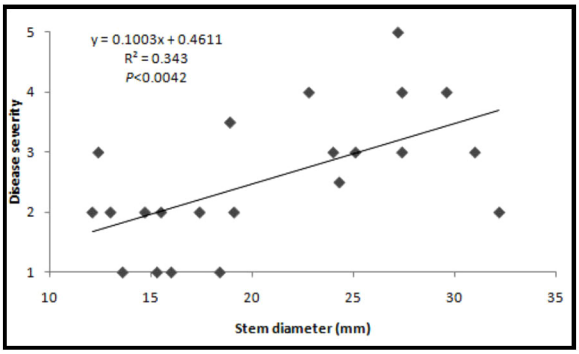

Figure 2. Relationship between plant size and internal and external development of laurel wilt symptoms on artifically inoculated Simmonds.

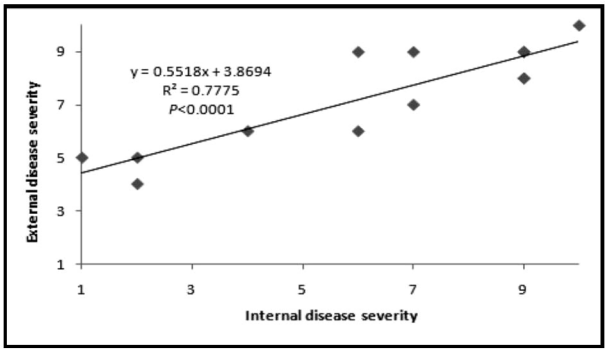

Figure 3. Relationship between severity of laurel wilt that developed on Simmonds artificially inoculated with *Raffaelea lauricola*.

Avocado responds to infection by *R. lauricola* by accumulating phenolic substances and producing tyloses in vessel elements, typical host defense responses (Figure 4). Studies are underway to distinguish macroscropic and microscopic reactions of susceptible and tolerant cultivars of avocado and other host species against this disease.

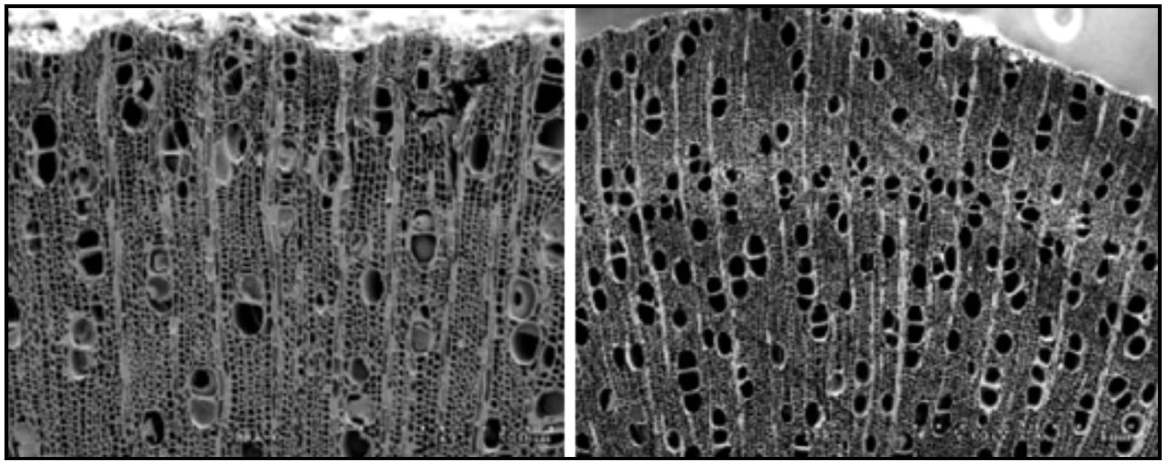

Figure 4. SEM images of 'Brogdon' avocado cultivar inoculated with *Raffaelea lauricola* (left) vs. mock-inoculated (right). Note tylose formation in xylem vessels in the image on the left.

Although macro-infusion (injection) has been used to effectively apply fungicides to tree vascular systems (Stennes 2000; Stipes 2000), and has been used to inject propiconazole in avocado and redbay (Mayfield et al. 2008a; Ploetz, unpublished data), the expense of macro-infusion would not allow it to be used cost-effectively in commercial avocado production (Evans, personal communication). Thus, other means of application are needed to achieve effective fungicide concentrations in the host infection court, xylem.

To this end, we examined soil drench and trunk applications of fungicides to manage this disease (Ploetz and Perez 2010). In general, triazole fungicides effectively inhibited disease development in artificially inoculated 'Simmonds' plants in greenhouse studies (data not shown). Minor internal symptom development occurred in propiconazole-treated plants; the pathogen could not be recovered at any site above or below the point of inoculation. Although soil drench and trunk sprays in Pentrabark solutions were both effective, drench application required about ten times more fungicide for comparable control. Current field experiments aim to develop means by which effective xylem concentrations of propiconazole could be achieved via trunk/branch applications in surfactants.

Despite good *in vitro* activity against the pathogen, azoxystrobin and fluazinam had no impact on disease development in the greenhouse work. Unfortunately, Agri-fos, which is labeled for avocado and has xylem and phloem mobility, was ineffective regardless of how it was applied.

Diagnosis and Interdiction

The SSU primers have enabled the sensitive detection and localization of *R. lauricola* in artificially inoculated plants, and have been valuable tools in studies on host:pathogen interactions, fungicidal control, epidemiology, and resistance (Dreaden et al. 2008; Ploetz et al. 2010; Ploetz and Perez 2010). A species-specific diagnostic tool, now under development, will play an important role in laurel wilt interdiction and laurel wilt management in avocado production areas via sanitation. Early detection of the pathogen and disease are critical to the success of sanitation efforts in new disease outbreaks (Ploetz 2007).

SUMMARY

Laurel wilt kills avocado, *P. americana*. The disease is an immediate threat to production in Florida, as well as the national clonal repository at the USDA/ARS station in Miami, Florida (Evans et al. 2010). As it spreads, other producers in the Western Hemisphere need to be aware of the problem. Germplasm should not be imported from affected areas unless its pathogen-free status can be confirmed. Diagnostic tools that have been and will be developed could play important roles in these certification efforts, and have been most useful in diverse studies of the disease and pathogen (Dreaden et al. 2008; Ploetz et al. 2010; Ploetz and Perez 2010).

Laurel wilt will be a difficult management problem. Fungicides, tolerant germplasm, sanitation, and various chemicals for managing the insect vector of the pathogen may ultimately all be useful when combating this important and destructive new disease.

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