Agri-dogs: Using Canines for Earlier Detection of Laurel Wilt Disease Affecting Avocado Trees in South Florida

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Summary. **The invasive redbay ambrosia beetle (*Xyleborus glabratus*) was first detected in Savannah, GA, in 2002. This tiny beetle and its symbiotic fungal partner (*Raffaelea lauricola*) have led to one of the most devastating new plant diseases in recent times affecting laurel trees (Lauraceae), laurel wilt. In Florida, this devastating disease has also affected the agriculturally important avocado (*Persea americana*), and once symptoms are visible (i.e., wilting leaves), it is too late to save the infected tree. However, prophylactic systemic treatment with propiconazole can protect the trees from the disease for ≈12 months. This study evaluated the novel approach of using scent-discriminating canines (*Canis familiaris*) trained on the volatiles of laurel wilt pathogen as a proactive management tool for grove owners. Canine deployments in groves resulted in the detection of 265 presymptomatic avocado trees during two trials. In trial 1, 155 presymptomatic trees were treated with propiconazole and, over the subsequent 14-month monitoring period, 97% remained asymptomatic. In trial 2, the canines detected 100 presymptomatic trees that were not subsequently treated and 22 progressed to wilt within 2–5 weeks, and the remaining trees were removed, thus halting the observation period at 6 weeks. The canines have proven to be an effective proactive management tool.**

Laurel wilt disease is a systemic wilt affecting laurel trees (Fraedrich et al., 2008; Mayfield and Thomas, 2006). This disease is incited by a fungal pathogen *R. lauricola*, which was introduced through its original vector, the redbay ambrosia beetle (Fraedrich et al., 2008; Hanula et al., 2008; Harrington et al., 2008; Mayfield and Thomas, 2006), and is now found associated with several native ambrosia beetle (Scolytinae) species (Ploetz et al., 2017c), some of which have been shown to be vectors (Carrillo et al., 2014). The original introduction in Georgia led to devastation and the loss of 300 million native woody laurel trees along the coastal forests from North Carolina to Florida (Harrington et al., 2011; Hughes et al., 2017) and spread rapidly in 15 years throughout Florida and as far west as Texas and Louisiana. In Florida, native laurel trees and the commercially important avocado are hosts of this lethal disease, which now poses a serious threat to the avocado industry (Crane et al., 2013, 2015; Ploetz et al., 2017a, 2017b). Current estimates attribute the loss of ≈25,000 avocado trees to laurel wilt since its migration into Florida (D. Pybas and J.H. Crane, personal communication).

Ambrosia beetles have highly specialized relationship with their symbiotic fungi. Adult female beetles harbor fungal symbionts within specialized sacs known as mycangia and inoculate host trees with their symbionts as they bore inside and excavate breeding galleries (Batra, 1967). *Raffaelea lauricola* subsequently colonizes and spreads through the xylem vessels, thus activating the tree's defense response. Host trees begin occluding xylem vessels in an attempt to quarantine and block the spread of the fungus (Inch and Ploetz, 2012; Inch et al., 2012). This usually is sufficient to stop the disease; however, in the case of laurel wilt disease, it appears that a hypersensitive reaction ultimately leads to the tree's death due to complete occlusion of the entire xylem and shutdown of the water transport system (Inch et al., 2012).

The management of laurel wilt has proven to be difficult. A healthy mature tree can be killed within 4–8 weeks after inoculation, and once symptoms are visible, it is too late to save the tree. Early symptoms of laurel wilt include wilting of part or the entire tree canopy, quickly followed by leaf desiccation and browning. As the disease progresses, stems and limbs dieback until eventually the entire tree dies. There is no fungicide presently approved capable of curing an infected tree, although propiconazole has been shown to provide around 12 months of protection when injected or infused prophylactically into trees (Mayfield et al., 2008; Ploetz et al., 2017d).

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.4047	acre(s)	ha	2.4711
29,574	fl oz	μL	3.3814×10^{-5}
0.3048	ft	m	3.2808
2.54	inch(es)	cm	0.3937
28.3495	oz	g	0.0353
1	ppm	$ng{\cdot}\mu L^{-1}$	1
1	ppt	$g{\cdot}L^{-1}$	1
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

The control and spread of the disease is further complicated as *R. lauricola* can be transmitted from tree to tree through root grafting among adjacent trees, as well as by other ambrosia beetles (Carrillo et al., 2014). If laurel wilt–affected trees are not detected and rogued quickly, the disease can spread rapidly to adjacent trees (Ploetz et al., 2017a, 2017b). This significantly increases any treatment or rogueing costs. If presymptomatic infected trees could be identified before symptoms are visible, these trees could be treated or removed immediately, thus reducing or eliminating the spread of the pathogen from tree to tree via root graft and beetle transmission. This could reduce control costs significantly and improve the efficacy of treating healthy avocado trees with fungicides adjacent to laurel wilt–affected trees. Although more effective fungicide treatments and disease-resistant avocado cultivars are long-term goals of ongoing research, the best defense available today is sanitation. Early detection of laurel wilt through canine detection can facilitate more effective sanitation efforts and help to reduce the root transmission of the pathogen.

Canines have an incredible sense of smell because of the large size and physical structure of the olfactory organs, density of neurons, and vast numbers of functional receptors (Craven et al., 2010; Furton and Myers, 2001). These factors make canines capable of detecting volatiles at concentrations as low as 1–2 ppt. Law enforcement agencies and the military have used canines in the detection of drugs, explosives, money, weapons, and missing persons (Furton and Myers, 2001). The U.S. Department of Agriculture (USDA) and Border Control have used canines to detect illegal food products, invasive plants, and pests such as spotted knapweed [*Centaurea stoebe* (Goodwin et al., 2010)], brown tree snake [*Boiga irregularis* (Savidge et al., 2011)], and desert tortoise [*Gopherus agassizii* (Cablk and Heaton, 2006; Nussear et al., 2008)] at the borders and ports of entry. Canines have also been used to detect various cancers (Godfrey, 2014; McCulloch et al., 2006; Moser and McCulloch, 2010) and even for the detection of mold (*Aspergillus* sp., *Penicillium* sp., *Stachybotrys* sp.), bed bugs (*Cimex lectularius*), and termites (*Reticulitermes* sp., *Coptotermes* sp., *Cryptotermes* sp., *Inscisitermes* sp.) (Brooks et al., 2003; Griffith et al., 2007; Pfiester et al., 2008). This study reports on the use of canines trained on the scent of the laurel wilt pathogen as an early detection tool of presymptomatic trees and as an additional tool for overall disease management. The potential for successfully treating presymptomatic, *R. lauricola*–infected trees with systemic fungicide to maintain tree health and production is discussed.

Materials and methods

Canine training. Two canines were consistently used throughout the duration of this investigation, Cobra, a Belgian Malinois, and One Betta, a Dutch Shepherd. These dogs had no prior training and were acquired through the United States K-9 Academy and Police Dog Training Center (Hialeah, FL). The training aids used in the study consisted of infected avocado wood placed in controlled odor mimic permeation system [COMPS (Furton and Harper, 2008)]. COMPS are specialized polymer bags that allow odor to escape at a constant rate and contain the biological or chemical agent within. The amount of plant material used was ≈15–20 g, was sealed within COMPS, stored in aluminum resealable bags (Ziploc®; SC Johnson, Racine, WI) and allowed to equilibrate at least 24 h before use.

Briefly, canine training was performed in sequential stages. First, the canines were evaluated for their hunt drive and desire to work for reward. This was done with simple fetch exercises allowing the canines to hunt and locate their favorite chew toy in tall grasses, followed by "hide-and-seek" exercises in trees. Canines were then rewarded with their favorite toy and allowed to play as a positive reinforcement tool. The second phase of training involved the use of a universal detector calibrant [UDC (Furton and Beltz, 2017)], which is a synthetic compound with an odor found not to occur in nature. This began the process of odor association with reward and was done through the use of stainless steel odor boxes and the association of the toy with the UDC training aid. Once the canine proved successful at these two phases, the final step involved the substitution of the UDC for the target odor, laurel wilt disease–infected avocado wood. This initial training process was completed in ≈1 month followed by reinforcement training a minimum of three times per week thereafter; canines were monitored and evaluated over the course of 1 year and 229 training sessions before grove deployments. The dogs were subsequently certified following the practices published by the Scientific Working Group on Dogs and Orthogonal Detector Guidelines.

Canine deployment. Nine commercial avocado groves with active laurel wilt disease in Homestead, FL, were used to deploy the canines. Trees ranged in age from 15 to 85 years and were spaced from 20 to 26 ft in-row and 20 to 25 ft between rows. The canine searches led to the identification of 165 trees as potentially positive for *R. lauricola*, but nonsymptomatic. Trees alerted to by both dogs during a deployment were tagged and roots were sampled for DNA testing to confirm the presence of the pathogen. One hundred and fifty-five of the 165 laurel wilt canine-detected trees were infused with propiconazole (Tilt®; Syngenta Crop Protection, Wilmington, DE) using the standard guidelines published by University of Florida (2015) researchers and performed by the grove owners or grove service companies. Ten trees were left untreated or removed per the decision of the grove owner. Subsequent canine deployments and monitoring of the health status of all detected trees were performed for 14–16 months. Any trees that succumbed to disease were noted.

Root sampling. Because of the nature of this disease and the fact that this study was done primarily in privately owned groves, sampling for *R. lauricola* by cutting through the bark and into the sapwood of the trees was deemed to be unethical. Physically damaged trees resulted in increased beetle recruitment, which could spread the disease. Thus, from trees showing no visible signs of disease, root samples were collected to determine disease transmission status. *Raffaelea lauricola* has been isolated from the roots of container-grown and field avocado trees (Hughes et al., 2015). Fifteen to 30 root sections ranging in length of 10–15 cm, diameter of 0.2–3.0 cm, and 0.5–6 ft from the base of the tree trunks were collected from the soil underneath the canopy of the trees identified by the canines.

The root samples were surface sterilized by washing and removing excess soil with water, followed by a series of washes using 10% bleach for 1 min followed by a 3-min soak in 70% ethyl alcohol. The samples were then homogenized with an electric grinder (Micro-Mill®; Bel-Art-SP Scienceware, Wayne, NJ) to generate a mixture of consistently small particles/chips. These samples were then used for DNA extraction or for fungal culturing. DNA extraction from homogenized root samples and fungal cultures was performed using an extraction kit (Fast DNA Spin Kit for Soil; MP Bio, Solon, OH) and agitator (FastPrep-24 System; MP Bio) per the manufacturer's protocols. Homogenized root samples were also used to isolate *R. lauricola* for DNA testing using a semiselective media of cyclohexamide streptomycin malt agar with additional antibiotics added (CSMA+) as described previously (Ploetz et al., 2011).

DNA analyses. Targeted sequences were amplified from DNA extracts using two previously described techniques. A fluorescein amidite (FAM)–labeled Taqman probe (Thermo Fisher Scientific, Waltham, MA) for a species-specific DNA region of interest within the large subunit of the ribosomal-encoding gene (28S rDNA gene) was used for quantitative polymerase chain reaction per previously published protocols (Jeyaprakash et al., 2014). Amplification was performed with a thermal cycler (MJ-Mini Opticon; Bio-Rad Laboratories, Hercules, CA). DNA extracted from fungal isolates cultured from roots was also amplified using a previously published multilocus microsatellite protocol (Dreaden et al., 2014). Briefly, each reaction contained 1 ng·μL^{-1} template DNA, 1X Immomix (Bioline, Taunton, MA), 0.4 μM of each primer (Integrated DNA Technologies, Coralville, IA), and sterile water to a final volume of 25 μL. Amplification parameters were initial denaturation step of 95 °C for 10 min, 40 cycles of 95 °C for 35 s, 65 °C for 30 s, and 72 °C for 30 s. Amplification products were separated on an Applied Biosystems 3130xl Genetic Analyzer using POP-7 separation polymer, Module DS 30, filter D, and GeneScan ROX 500 internal size standard (Thermo Fisher Scientific). The injection was for 23 s and subsequent analyses used Applied Biosystems GeneMapper® software (version 3.7, Thermo Fisher Scientific).

Grove monitoring for laurel wilt. An observational trial was conducted within a 40–50-year-old 25-acre grove where several hundred trees were visibly affected with or had been removed because of laurel wilt. The owner opted not to treat any trees in the grove with systemic fungicide and instead decided to remove all trees. Before the grove was to be bulldozed, the owner granted permission to deploy the canines and monitor trees for disease until the grove was bulldozed. Two canines detected 100 presymptomatic trees out of an ≈800-tree area in the grove within 1 week (Fig. 1). The trees were tagged and their global positioning system (GPS) coordinates recorded. Trees were then routinely checked biweekly for visible symptoms (e.g., wilting, leaf desiccation, stem dieback, etc.) of the disease. Because the grove was known to be affected by laurel wilt, DNA testing was not a priority as there is no other known avocado disease that under dry soil conditions in south Florida kills avocado trees (J.H. Crane, personal communication). The study was run for a period of 6 weeks before the grove was bulldozed; because of the time constraint, the fate of all 100 trees could not be followed.

Economic analysis. A simple economic feasibility analysis was performed through calculating the costs of canine deployment and treatment of presymptomatic trees and comparing that cost with the value of a mature fruit-bearing avocado tree. This analysis was carried out using an interactive Tree Value Calculator tool from the agricultural economics extension website at the University of Florida (Evans, 2009; Evans and Crane, 2016). The analysis model had the following assumptions: assume a 5-acre canine search area, fruit-bearing trees are 8 years or older, detected trees showing no visible signs of disease, and successful professional treatment of trees with propiconazole was carried out within 5 d of canine detection. In addition,

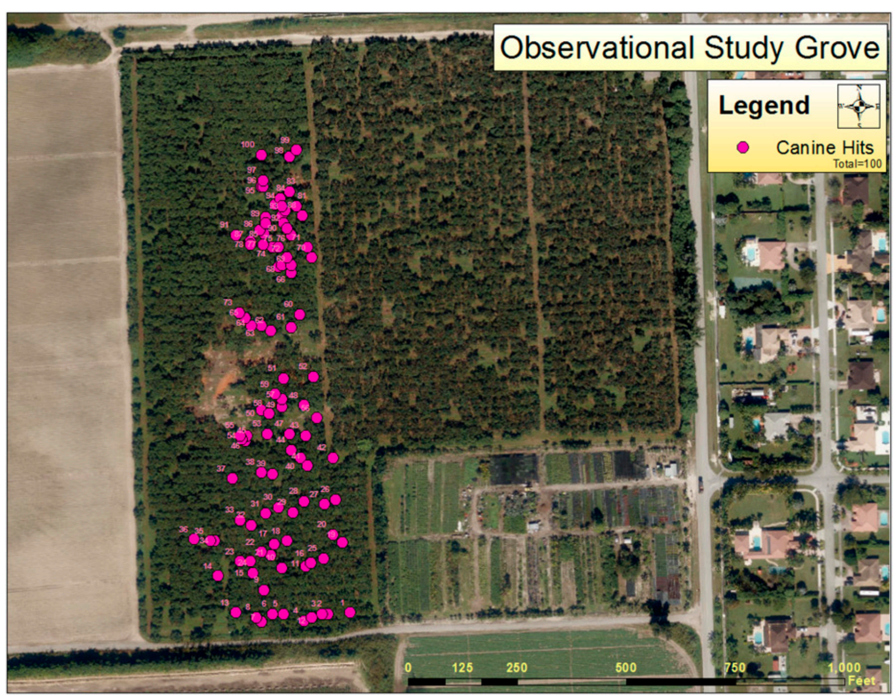

Fig. 1. A map showing the avocado tree canines alerted to as presymptomatic for the laurel wilt pathogen during trial 2. Trees were visually monitored for 6 weeks before the grove was bulldozed. Of the 100 trees with canine alerts, 22 trees progressed to laurel wilt with all the visible symptoms and nine more showed early signs of the disease before the grove was bulldozed.

follow-up monitoring and, at the grove owner's discretion, fungicide treatments of the disease hot spots every 12–18 months must be performed to maintain protection of trees.

Results and discussion

After more than 229 training sessions with three canines, Cobra (73), OneBetta (78), and Candy (78), it was found that the average time to alert was 40.82, 37.43, and 48.50 s, respectively. The average accuracy and positive predictive value was 99.4% and 94.8% (Table 1).

TRIAL 1. During the course of this study, the certified scent-discriminating canines were deployed to nine commercial avocado groves affected by laurel wilt disease. Two canines were deployed in each grove and the canines alerted to 165 trees that were potentially positive for *R. lauricola*; these trees were classified as presymptomatic for laurel wilt—no visible disease symptoms. Only trees that both canines independently alerted to in separate deployments were considered positive alerts. Of these 165 trees, 155 were subsequently treated with propiconazole within 1–5 d of canine alert and, over a monitoring period ≥14 months, 151 of these trees remained healthy (97%) with no visible disease symptoms. Four trees succumbed to laurel wilt 1 year after the initial treatment. Ten trees that were potentially infected by *R. lauricola* (i.e., canines alerted) and were not treated (decision of the grove owner). Of those, seven of them succumbed to laurel wilt (70%) and three trees (30%) were removed before symptoms could be monitored.

DNA detection indicated that of the 165 trees, the canines alerted to 95 (58%) tested positive for *R. lauricola,* using the 28S probe on DNA extracted from the roots. In addition, seven samples were positive using microsatellites and sequenced to confirm identity for isolates grown in culture (Table 2). Because the laurel wilt pathogen is unevenly distributed within trees, sampling roots of the detected trees may not indicate the presence of the pathogen (i.e., false negative) (Crane, 2013; Hughes et al., 2015; Inch et al., 2012; Ploetz et al., 2011) if the fungus had not infected the roots at the time of sampling. These data suggest that prompt fungicide treatment of presymptomatic but *R. lauricola*–positive trees allows the maintenance of health and production providing that follow-up treatments are applied as recommended (Tables 2 and 3).

TRIAL 2. This deployment resulted in the canines identifying 100 presymptomatic trees, of which 22 progressed to full wilt within the 6-week observational period. An additional nine trees showed signs of early disease (i.e., wilting of leaves). Four trees progressed to wilt within 2–3 weeks, whereas the remainder of the 22 began showing signs of disease 4–6 weeks postcanine alert (Fig. 2). Unfortunately, all 100 trees could not be observed before the complete grove was bulldozed and all trees destroyed.

LAUREL WILT MANAGEMENT POTENTIAL. Out of the nine groves surveyed in trial 1, three owners specifically chose to follow all recommended laurel wilt sanitation guidelines (Crane, 2013) based on the canine alerts. This included the immediate removal of laurel wilt–affected trees, chipping and burning the wood followed by the systemic treatment with propiconazole of a minimum of two to three rings of healthy trees surrounding the removed tree and disease foci. Over the course of this investigation, 151 of the 155 canine-alerted, presymptomatic trees were treated with propiconazole. These 151 treated trees remained healthy over a 14-month period. After 14 months, only four trees in grove 1 began showing signs of wilt (Table 4).

ECONOMIC ANALYSIS. The calculations indicate a potential savings per 5 acres ranging from $300 to $4350, depending on the number of presymptomatic trees detected and treated (Table 5).

Conclusions

Invasive species because of their very nature tend to be major problems in the non-native ecosystems they invade. With extensive global trade and travel, the rate of new introductions of insects and diseases has continued to grow and has become a significant problem (Batra, 1967). A naïve host, when faced with a novel pathogen, is at a great disadvantage, and if the host is of significant economic or ecological value, the impacts on the ecosystem and commercial interests can be catastrophic (Griffith et al., 2007; Ploetz et al., 2013). Generally, indigenous and exotic ambrosia beetles are not significant environmental or agricultural pests (Ploetz et al., 2013). In their native habitats, they usually attack dead and decaying wood, or compromised trees. But recent ambrosia beetle introductions have become invasive and the redbay ambrosia beetle attacks healthy trees possibly because of olfactory cue mismatch with the host tree (Hulcr and Dunn, 2011; Ploetz et al., 2013). To date, laurel wilt disease has killed nearly a half billion native laurel trees throughout the southeastern United States and in many cases, has completely restructured critical ecosystems and coastal forests. To add to the problem, the disease has spread to the agriculturally important avocado production area of south Florida. Other ambrosia beetles

Table 1. Summary results of the canine searches for training aids (controlled odor mimic permeation system bags with laurel wilt–infected avocado wood) during the time period of Sept. 2014 to Apr. 2015. There was no significant difference between the canines in terms of average time to alert correctly to a training aid (analysis of variance $P > 0.05$, df = 2, $F = 2.04$). The average accuracy (ACC) of all three canines [Candy (78), Cobra (73), and OneBetta (78); $n = 229$] was ≈99.4%.

Canine name	Canine breed	Avg time to alert (s)	Failures to alert (no.)	False alerts (no.)[z]	ACC/PPV (%)[y]
Candy	Dutch Shepherd	48.50	10	0	99.5/100
OneBetta	Dutch Shepherd	37.43	1	10	98.9/87.0
Cobra	Belgian Malinois	40.82	2	2	99.7/97.3

[z]False alerts indicate when a canine sits on a tree that does not hold a training aid.
[y]ACC is calculated as the true-positive alerts + true negative alerts divided by the total positives trees + total negative trees; positive predictive value (PPV) is calculated as the true-positive alerts divided by the sum of the true-positive alerts and false-positive alerts.

Table 2. Laurel wilt DNA results for avocado tree canines identified as presymptomatic for laurel wilt disease using root samples.

Trees detected (no.)	DNA positive for *Raffaelea lauricola* (no.)[z]	Large subunit probe positive for *R. lauricola*	Microsatellite PCR positives of isolates grown in culture
165[y]	95	92	7 (4 + 3)[x]

[z]DNA results displayed show the total number of large subunit probe polymerase chain reaction (PCR) (direct from root extraction) and microsatellite positives (from isolates).
[y]Trees were sampled for *R. lauricola* before fungicide treatment.
[x]Four samples were confirmed positive for *R. lauricola* with microsatellites after culturing from root samples and an additional three samples were negative for the probe but positive on culturing.

Table 3. The number of commercial avocado tree canines detected as laurel wilt suspects, number of suspect trees treated with propiconazole fungicide, and number of trees dead 14 months after fungicide treatment.

Trees canines detected (no.)	Infusion treatment	Healthy trees (no.)[z]	Healthy trees (%)[z,y]	Trees progressed to wilt (no.)	Trees progressed to wilt (%)[y]
155	Treated with propiconazole	151	97	4	3
10	Nontreated	3	30	7	70

[z]After 14 months of observation.
[y]Calculated numbers rounded to nearest integer.

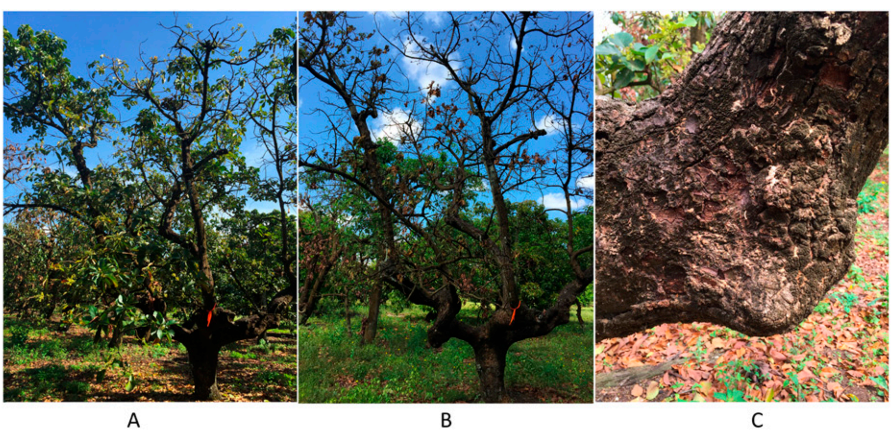

Fig. 2. Image taken of an avocado tree in a commercial grove at the time of the canine alerts (A), and 2 weeks postcanine alert (B and C), showing symptoms of laurel wilt during trial 2.

Table 4. Disease management results for three individual avocado groves where grove owners followed recommended prophylactic propiconazole treatment and guidelines based on canine alerts.

Grove no.	Trees in grove (no.)	Presymptomatic trees detected by canines (no.)	Treated trees (no.)	Treated trees that progressed to wilt (no.)	Protection period (mo.)
1	2,250	35	342	4	14
2	1,200	49	281	0	16
3	1,000	23	62	0	9

attacking an infected tree have picked up *R. lauricola* as a contaminant and are now thought to be the primary vectors for laurel wilt in commercial avocado groves (Carrillo et al., 2014). This extends the problem beyond an ecological disaster and now affects the economy of the state of Florida and has the potential to be the causal agent of thousands of job losses. This industry generates $30 million to $54 million per year (Evans et al., 2010; Ploetz et al., 2011) and the economic impact of this disease on the avocado industry is estimated to be $400 million (Evans et al., 2010). The rapid spread of this disease could result in an even greater disaster should it reach Mexico, California, and Central and South America.

Historically, other well-studied tree diseases such as chestnut blight (*Cryphonectria parasitica*) and Dutch elm disease (*Ophiostoma* sp.) are similar to the laurel wilt. Chestnut blight incited by a fungal ascomycete, *C. parasitica*, which originated from a shipment of nursery trees from Asia (Gruenwald, 2012). It was discovered that there were two forms of the pathogen, one possessing viral RNA sequences that lead to a loss of pathogenicity. This phenomenon, termed hypo-virulence by attenuation (Nuss, 1992; Peever et al., 2000), lead to the successful biocontrol of the disease. Dutch elm disease was incited by another invasive fungus vectored by a bark beetle and similar to laurel wilt, also originated from Asia (Brasier, 1991; Harwood et al., 2011). The management of this disease involved pruning trees and removing debris for burning and sanitation, followed by the infamous use of DDT, a pesticide used to combat beetles that subsequently had devastating effects on the environment (Harwood et al., 2011). Fungicides as well as beetle traps were used. Resistance to the disease through biological agents was successful where a species of nonpathogenic fungi was applied to trees to stimulate immunity and resistance. Finally, the best scenario for management of tree diseases is development of resistant cultivars; however, this is a long-term approach. Indeed, the breeding programs leading to hybrids have produced disease-resistant elms (Harwood et al., 2011).

What can be learned from these classic examples is that management of new invasive tree disease is a daunting task and a costly one. Chemical control without due diligence can lead to unintended consequences, and although biological control and breeding programs for disease-resistant trees are ideal, they require significant research and funding and usually are long-term solutions. The case of laurel wilt is not different, and the best solution available at the current time involves reactive vs. proactive approaches.

In an effort to provide a more rapid response and aid the farmers in this difficult situation, scent-discriminating

Table 5. Cost and a simple economic feasibility analysis of canines as an early detection tool for laurel wilt disease in a 5-acre (2.0 ha) commercial grove.

Activity	1 tree	2 trees	3 trees	4 trees	5 trees	10 trees
Canine deployment[z]	$150	$150	$150	$150	$150	$150
Fungicide treatment[y]	$25	$50	$75	$100	$125	$250
Sum	$175	$200	$225	$250	$275	$400
Value of saved trees[x]	$475	$950	$1,425	$1,900	$2,375	$4,750

[z]Cost to deploy canines on a 5-acre grove.
[y]Cost of infusion with propiconazole fungicide.
[x]This simplified analysis sets deployment costs at $150 for 5 acres. The value of a lost tree is calculated using the University of Florida Agricultural Economics Extension interactive Tree Value Analysis tool. Assumptions and factors considered are the age of the bearing tree (mature at 8 years or more), interest rates, the farm gate price of avocado fruit per pound, the cost of stump removal if tree succumbs to laurel wilt, and the replant costs of a new tree.

canines were trained on the scent of *R. lauricola* and deployed in several groves to determine their ability to improve rapid disease detection and subsequent management. It appears that visually nonsymptomatic but *R. lauricola*–infected avocado trees, if detected before visual disease symptoms (i.e., wilting, desiccation, etc.) and immediately treated with systemic fungicide, may survive for at least 14 months. This approach represents a significant leap in the proactive monitoring and early detection capabilities of the farmer. The goal of early detection and treatment with canines is to significantly reduce the spread of laurel wilt outbreaks, delineate the hot spots of the disease, and to reduce the costs to the grove owner.

This study is the first documented investigation to evaluate the use of canines to detect a xylem-based vascular wilt disease in trees. Ongoing work at the USDA has used canines to detect citrus canker (*Xanthomonas axonopodis*) and reports high accuracies of ≈98% (USDA, 2010). Previously published studies have demonstrated good accuracy and capabilities with canines used in the conservation of animal species such as the desert tortoise and brown tree snake (Cablk and Heaton, 2006; Nussear et al., 2008; Savidge et al., 2011) and also demonstrated an ability to outperform human surveyors in locating the rare spotted knapweed, a problematic invasive plant (Goodwin et al., 2010).

During the course of this study, an opportunity was presented to monitor the visual progression of presymptomatic trees in a private, laurel wilt–affected grove (trial 2). This study resulted in the detection of 100 presymptomatic trees within a 3-d period, and although the grove was bulldozed after only 6 weeks of monitoring, 22 trees with positive canine alert progressed to wilt and an additional four demonstrated some sign of possible wilt with green leaves beginning to wilt. Nonetheless, the results suggest that the canines have the ability to alert to *R. lauricola*–infected trees as far as 40–46 d before any visual symptoms developed.

Finally, of the nine groves where canine deployments were routine (trial 1), three owners followed the recommended guidelines for sanitation and also treated presymptomatic trees identified by the canine alerts plus one to two rings of trees around the infected tree with propiconazole (Table 4). These three groves were all affected by chronic laurel wilt infections before the canine detection and systemic application of propiconazole to these presymptomatic trees. Over the period of 14 months, canine alerts lead to the treatment of 342 (≈15%) trees in grove 1, 281 (≈23%) in grove 2, and 62 (≈6%) in grove 3. Of the trees treated, only four succumbed to wilt and only did so after 14 months after the initial treatment with propiconazole in grove 1. This demonstrated the importance of being vigilant and early treatment but also showed the necessity to possibly redeploying the canines to monitor even after fungicide infusions and perhaps the need for repeated treatment to maintain protection. More importantly, these three groves demonstrate the validity and success of using canines as a management tool specifically for early detection. It is of special importance that grove 1 had lost in excess of 100 trees to wilt before this approach and only four since deployment (after 12+ months posttreatment). Also, the cost of treating every tree in a grove is prohibitive for the vast majority of growers and this approach resulted in successful management with only proactively treating hot spots or a small fraction of the total number of trees.

To further evaluate the financial benefits of early detection, an economic analysis was carried out. The cost of deployment for canines was set at $150 for 5 acres (≈500 trees) and the cost of treatment at $25 per tree. Because management of this disease is costly, many grove owners opt to "do nothing" to minimize the spread of the disease. Economically, to prophylactically treat an entire grove with propiconazole is cost prohibitive for many. Therefore, a cost/benefit evaluation was performed as a guide for the grove owners and managers to use. To inform them about the immediate costs of canine detection and subsequent treatment of a presymptomatic tree, that could save it. This could then be weighed against the value of that tree (capital asset) if it was lost. Calculations and projections were performed using data obtained through the Food and Resource Economics Department, at the University of Florida (De Oleo et al., 2014). The result indicated that early detection has the potential to avoid losses ranging from ≈$300 to $4350, depending on the number of trees identified. The ability of canines to detect these trees before symptoms greatly enhances the ability to stop the spread of infections, reduces losses, and saves money over the long term. It is also important to note that when a tree is lost and a new one planted, it takes 6 years before it bears fruit and a total of 7 years until they are considered mature (De Oleo et al., 2014). Even then, that tree will likely not produce more than half of the product as the previously lost older tree. Therefore, this type of proactive approach using canines is an economically worthy effort in the fight against laurel wilt disease. Canine detection provides a rapid response to managing the spread of infection and proved to be cost effective in both the short term and the long run. World trade today has no physical borders and thousands of miles of ocean are irrelevant with respect to the movement and establishment of invasive species. Global commerce and trade has led to the introduction of devastating diseases and disturbances to native and naïve ecosystems (those not previously exposed to new diseases), and the frequency of these events is only expected to increase. The options for management and protection are often very limited because of the simple fact that, by nature, these events are spontaneous and new. A common factor in the treatment of any disease is that early detection will significantly improve the prognosis and the potential to prevent, treat, or reduce the spread. Therefore, innovative and rapid deployment methods are key. Canines provide a highly sensitive biodetector system for virtually anything with an odor, and this study demonstrated their capabilities as an additional management tool in an agricultural setting. In conclusion, this study demonstrated that canines can be critical as an early detection tool for the monitoring of both new and existing diseases and pests of important commercial crops. In the case of laurel wilt, being able to detect up to 46 d before symptoms appear, it opens a window to save trees that previously would have been lost.

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