An Evaluation of Scent-discriminating Canines for Rapid Response to Agricultural Diseases

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SUMMARY. Laurel wilt disease, incited by Raffaelea lauricola, has resulted in the death of more than 300 million laurel trees (Lauraceae) in the United States. One such tree is the commercially important avocado (Persea americana), the second largest tree crop in Florida other than citrus (Citrus sp.). This disease affects the industry in South Florida and two larger avocado industries in Mexico and California have taken notice. Trees succumb soon after infection, and once external symptoms are evident, the disease is very difficult to control and contain as the pathogen can spread to adjacent trees via root grafting. Presently, there is no viable, cost-effective method of early diagnosis and treatment. This study was undertaken to evaluate the use of scent-discriminating canines (Canis familiaris) for the detection of laurel wilt–affected wood from avocado trees. Three canines, one Belgian Malinois and two Dutch Shepherds, were trained and studied for this ability. In addition, prevailing weather conditions were recorded and evaluated to determine their effect on canine performance. The results of this evaluation indicated that canines can detect laurel wilt–affected wood and the laurel wilt pathogen and may be useful in the detection of laurel wilt–diseased trees in commercial groves.

Laurel wilt is the consequence of an invasive species, the redbay ambrosia beetle (RAB) (Xyleborus glabratus) introduced into the United States in untreated wooden packaging material. It was first detected in diseased redbay trees (Persea borbonia) in Savannah, GA, in 2002 (Mayfield and Thomas, 2006). The beetle, originally from Asia, was found to be the vector of the disease in redbay trees and was thus named RAB (Fraedrich et al., 2008; Hanula et al., 2008; Mayfield and Thomas, 2006). The term ambrosia refers to an ecological relationship that these beetles share with fungal partners. These partners have coevolved to become dependent on one another for survival and dispersal. Ambrosia beetles belong to the subfamily Scolytinae and are some of the most common and the most devastating pests to plants known to date (Ploetz et al., 2013). Although its original introduction was through the RAB, it is important to note that in avocado, multiple beetle vectors have demonstrated the ability either as a contaminant or a new vector to inoculate trees (Carrillo et al., 2014).

Ambrosia beetles typically harbor fungal spores within specialized sacs known as mycangia, which excrete fungal spores as they bore into host trees, thus inoculating the tree with the fungus. The beetle then actively cultivates or farms the fungal gardens within excavated galleries as a food source for itself and its developing larvae (Batra, 1967; Ploetz et al., 2013). In laurel wilt disease, this particular fungal partner was identified as a lethal pathogen R. lauricola (Fraedrich et al., 2008; Harrington et al., 2008). The name, laurel wilt disease, was coined when the fungal spores of R. lauricola were shown to infect and lead to wilt symptoms in many species of laurel trees (Harrington et al., 2008), including the commercially important avocado (Ploetz et al., 2017a). This is a systemic disease incited by the clogging of the xylem vessels through the tree’s production of tylose-, phenolic-, pectin-, and lipid-containing gums or gels as it tries to defend itself from the systemic infection (Inch et al., 2012), a general response that can be produced because of several host insults. The result of blocked xylem vessels is compromised water and nutrient flow during evapotranspiration, visible wilting of the leaves, tissue necrosis, and, ultimately, the death of the tree. The presence of the fungus can be indicated visually through black/brown-stained sapwood, caused by the host response (Fraedrich et al., 2008; Inch and Ploetz, 2012; Inch et al., 2012).

Laurel wilt disease is highly aggressive and it has been demonstrated that only a few spores (as low as 100 conidia) or a single beetle-boring event can be sufficient to elicit the systemic disease in avocado. More than 300 million wild laurels have been lost and presently laurel wilt disease is rapidly spreading through the South Florida avocado groves, a $54-million/year industry (Evans et al., 2010), with the cost of replacement of trees rising to $400 million (Evans et al., 2010).
economical and ecological disaster will be even greater if this disease infects the larger avocado production areas such as California and Mexico.

Presently, the fungicide propiconazole (TILT®, Syngenta Crop Protection, Wilmington, DE) that suppresses the growth of the fungus but does not kill it is used to protect trees from infection. This formulation can provide 11–12 months of protection depending on the method of administration into the trees (Ploetz et al., 2017a, 2017b, 2017c). However, to prophy-lactically treat all trees comes with a high cost that many small farmers simply cannot afford. Studies geared toward early detection of laurel wilt disease using aerial image and remote sensing with drones have demonstrated promise in distinguishing between laurel wilt–affected trees from healthy trees as well as other stressors (de Castro et al., 2015a, 2015b). To aid in the early detection and control of this disease, scent-discriminating canines have been trained on laurel wilt–affected avocado wood to enable earlier detection and a more focused hot spot treatment that provides a cost-effective treatment plan for farmers.

Presently, canines are extensively used in law enforcement and forensics in the location of missing people, explosives, drugs, weapons, and ammunition (Furton and Myers, 2001). Furthermore, canines have demonstrated the ability to detect invasive species of spotted knapweed [Centaurea stoebe (Goodwin et al., 2010)], brown tree snake [Boiga irregularis (Savidge et al., 2011)], desert tortoise [Gopherus agassizii (Cablk and Heaton, 2006; Nussear et al., 2008)], and various cancers (Godfrey, 2014; McCulloch et al., 2006; Moser and McCulloch, 2010). They have even been used to detect bed bugs [Cimex lectularius], termite [Reticulitermes sp., Coptotermes sp., Cryptotermes sp., and Incisitermes sp.], and mold [Aspergillus sp., Penicillium sp., and Stachybotrys sp.].

This study was conducted in a privately owned mango (Mangifera sp.) grove in the Redland agricultural district near Homestead, FL, under the Institutional Animal Care and Use Committee (IACUC) approval (IACUC-15-011-CR01). No laurel wilt was detected in the immediate vicinity.

**Canine selection and training aids**

Canines with no prior training were acquired through the United States K-9 Academy and Police Dog Training Center in Hialeah, FL (Fig. 1), and trainers and handlers were provided with training aids. The training aids consist of laurel wilt–affected avocado wood as well as the fungal pathogen R. lauricola 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 but contain the biological or chemical agent within (Fig. 2). The amount of plant material used was ≈15–20 g, was sealed within COMPS, stored in re-sealable aluminum envelopes and allowed to equilibrate at least 24 h before use.

**Canine training**

The training of the canines took place in three main phases.

**Phase 1.** Using an open outdoor area of “tall grasses,” the dogs were evaluated for their “hunt drive.” This phase involved simple fetch exercises with a chew toy to determine whether they had the desire to search for extended periods of time for the reward of playing with the toy.

**Phase 2.** This phase involved the use of stainless steel odor boxes and a universal detector calibrant [UDC...
and other distractors that seemed to affect the canine’s accuracy in an agricultural setting were recorded.

**Evaluating the effect of weather conditions on canine performance**

Temperature, relative humidity, and wind speed were recorded at the start of each canine deployment using a weather monitor (Kestrel 4500; Loftopia, Birmingham MI).

**3-D controlled study**

A study was conducted to evaluate whether canine performance was influenced when both the handler and the canine were blind to the placement of the training aids (simulation of a real deployment scenario). Within the same mango grove, a training area consisting of 25–30 trees was designated and each tree was assigned a number. COMPS training aids with healthy or laurel wilt–affected avocado wood were prepared and the selection of trees in which to hide/place the training aids was performed using a random number generator. In each run, three positive aids and one negative control (healthy wood) were used. The evaluator then walked up and down each row, stopping at and touching each tree to ensure that the canines did not track the human scent but rather were searching for the training aid. After 5 min, the handler teams worked with their canines to locate the hidden aids, and the time to alert on a positive aid was recorded. On each of the three different days, training aid detection was evaluated at 9:00 AM, 1:00 PM, and 4:00 PM, with new random placements of the training aids for each run.

**Pathogen detection and negative control study**

Training aids with only the laurel wilt pathogen, grown in pure culture and obtained through University for Florida Tropical Research and Education Center (UF-TREC) pathology laboratory (Ploetz et al., 2011), were placed in sealed COMPS bags to determine if the canines were able to successfully detect and alert to just the fungus. All materials such as empty COMPS bags as well as the media used to grow the pathogen were also set out as distractors during a 3-d trial (conducted at a different time to the previously described 3-d trial). A “positive pathogen” aid was placed within the training area, as well as empty COMPS bags and one growth media–only sample sealed in a COMPS. The dogs were then deployed, and observations were made and alerts were recorded.

**Canine/handler certification**

Performance of the canine handler teams was evaluated with independent evaluators (not otherwise involved with the study) following the approved best practice guidelines of the Scientific Working Group on Dogs and Orthogonal Detector Guidelines (SWGDog), which specify that dogs respond with a minimum of 90% positive rate and a corresponding maximum false alert rate of 10% (Furton et al., 2010).

**Results**

**Training evaluation.** A total of 229 training sessions with three canines, Cobra (73), OneBetta (78), and Candy (78), were performed and the search-locate-alert time for canines was recorded. The averaged time to alert was 40.82, 37.43, and 48.50 s for Cobra, OneBetta, and Candy, respectively. There was no significant difference between the times between canines [analysis of variance (ANOVA) $P > 0.05$, $df = 2$, $F = 2.04$]. There were a total of 12 false alerts in which the canines alerted to a tree not holding a positive training aid, 10 of which were attributed to OneBetta. There were also 12 failures to alert recorded, in which the canines did not locate the positive training aid during the session, with 10 being attributed to Candy. To evaluate canine performance, the accuracy (ACC) and positive predictive value (PPV) for each canine was calculated. The average ACC and PPV were determined to be 99.4% and 94.8%, respectively (J. Mendel, C. Burns, B. Kallifatidis, E. Evans, J. Crane, K.G. Furton, and D. Mills, unpublished data).

**Effect of weather on performance.** Scatterplots with trend lines (Figs. 3–5) demonstrated shorter times to target location associated with temperature and wind speed, compared with longer search times associated with higher humidity. Multiple linear regression analysis revealed an $R^2$ of 0.07, with the only significant variable being temperature ($P < 0.05$).
It was observed during the study that false alerts were most often observed on trees downwind but near to the target tree. The analyses found that, with the exception of two alerts, false alerts were all observed when wind speeds were about 5 km·h⁻¹ or higher (Fig. 6).

**Controlled Studies.** A 3-d controlled study demonstrated high accuracy and precision by all three canines, and ANOVA results showed no difference in the time to alert during different times of the day for deployment \( P > 0.05, \text{df} = 2, F = 3.47 \) (Table 1). When compared with the 229 canine training sessions, it was observed that the randomized, double-blind study led to significantly longer search times \( P < 0.05, \text{df} = 1, F = 5.10 \) (Table 1). The second controlled study involving a variety of negative controls led to only one false-positive alert on growth media by OneBetta (Table 2).

**Discussion**

This study demonstrated the ability of canines to detect laurel wilt. The goals of this study were to evaluate the success of canine training over an extended period, during the time period of Sept. 2014–July 2015 in Homestead, FL. With an average time to locate a positive sample of affected avocado wood that ranged between about 37–49 s with the canines working with about 25–30 trees in the study area, they proved to be not only an accurate detection option but also a truly rapid one. The high accuracy observed in this study was comparable to a study involving the detection of tethered tortoises (Cablk and Heaton, 2006), which boasted >90% accuracy in locating both surface and burrowed animals. Another study, with the same species of desert tortoise, evaluated detection accuracy in the wild with free-roaming tortoises than those tethered to a specific location. They reported 70% accuracy (14).

A low number of false alerts, only 12, were detected and interestingly, OneBetta was the canine responsible for 10 of those 12 alerts. This highlights an important point that each animal’s behavior differs. It was noted by the handler and the trainer that OneBetta has a tendency to be eager to alert as soon as she picked up the odor cone, but on denial of her reward, she then moves on to locate the point source. Failures to locate a training aid during a deployment were also noted. A large number of these failed deployments, 11 of them, were due to fallen fruit in the grove, which distracted the canines and resulted in long and interrupted search times and were early in their training. Other distractions that resulted in longer times and failed deployments included the presence of other wild animals being flushed from the grasses that distracted the canines from their searches. Fine-tuning of obedience training proved to reduce these incidents over the course of the training but highlights important factors to consider when deploying canines in an agricultural or uncontrolled environment. In Guam, a study involving the location of brown tree snakes, reported a low 35% success accuracy for the canine teams (Savidge et al., 2011). Perhaps, the lower accuracy in that study can be attributed to the added difficulty in locating moving targets. Another study involved the detection and location of an invasive plant species of knapweed. This small plant can be difficult to locate in bushy understory. The study made a direct comparison between handler canine teams and human-only surveyors (Goodwin et al., 2010). An overall canine accuracy of 81% was observed and canine teams outperformed human-only teams in their ability to locate the elusive plant.

The present study also calculated the PPV or precision of canines in their detection of laurel wilt–diseased avocado wood. The results ranged between 87% and 100% for the three canines. The high accuracy and precision in more than 229 deployments coupled with the design of the training, using infected avocado wood and training in an agricultural setting, strongly supports the viability of using canine detectors for laurel wilt disease detection.

The results of the controlled study in which both handlers and canines were blind to the placement of training aids had an average time to alert of about 58 s which was found to be significantly higher, using a Student’s \( t \) test \( P < 0.05 \), than the average of the 229 previous canine
deployments (about 42 s). However, because of the substantially smaller number of deployments in this experiment, it is unclear whether this was truly due to the additional controls and precautions being taken to mask the human trail or whether the difference would be significant with a comparable number of trials. During the controlled study, no alerts to healthy avocado wood COMPS were observed and in addition, no false alerts were observed to the blank COMPS or other distractors. There was no significant difference found using ANOVA between the accuracy of the three canines or in deployments taking place at different times of the day ($P > 0.05$).

Another goal of this study was to investigate the effect, if any, on canine performance in the often-harsh conditions such as high temperatures and humidity in South Florida’s agricultural environments. A previous study by Nussear et al. (2008) examined whether the prevailing weather conditions (wind speed, temperature, and relative humidity) affected the search times in canine handler teams in locating desert tortoises and found no relationship. Savidge et al. (2011) in the detection of snakes demonstrated increased success with decreasing wind speeds and increasing humidity. In an attempt to evaluate the predictive power of these variables on the time to alert, a linear regression analysis was done and demonstrated a weak model with an $R^2$ value of 0.07; however, temperature had a significant probability value (ANOVA) indicating that it possibly has a greater effect on odor dispersal from COMPS allowing for faster detection times. It is known that high temperatures can adversely affect canines (Furton et al., 2010), so perhaps, the small search area combined with higher temperatures led to greater odor dispersal and thus the faster detections observed. It is clear, however, that the canines are capable of performing at high levels under all the conditions observed during the course of the study. Interestingly it was observed that a higher number of false alerts were seen at higher wind speeds [$>4.8$ km·h$^{-1}$ (Fig. 6)], particularly for OneBetta who was responsible for 10 of the 12 false alerts over the course of the study. False alerts were usually on a tree downwind of the target tree. Only two false alerts were observed at low wind speeds. It is known that wind can transport odors making higher winds ideal for tracking and location over long distances (Cablak and Heaton, 2006); however, it can also result in pooling of odors away from the source and dispersal can make it difficult for the

### Table 1. A summary for the controlled 3-d evaluations of canine performance in locating laurel wilt training aids. Analysis of variance results indicated no significant difference between canines as well as no significant difference between time of day ($P > 0.05$, $df = 2$, $F = 3.47$) with respect to time to alert ($T_1$, $T_2$, and $T_3$). However, comparing these results to the 229 routine training results described in the text showed a significantly increased time to alert when both the handlers and canines are blind to the location of the training aid ($P < 0.05$, $df = 1$, $F = 5.10$).

| Canine name | Training time | $T_1$ (s) | $T_2$ (s) | $T_3$ (s) | Avg (s)$^a$ |
|-------------|---------------|-----------|-----------|-----------|-------------|
| Candy       | D1: 9:00 AM   | 43.54     | 45.01     | 71.2      | 53.25       |
| Candy       | D2: 1:00 PM   | 60        | 50        | 43        | 51.00       |
| Candy       | D3: 4:00 PM   | 150       | 25        | 32.9      | 69.30       |
| Cobra       | D1: 9:00 AM   | 85        | 63        | 169       | 105.67      |
| Cobra       | D2: 1:00 PM   | 40        | 43        | 80        | 54.33       |
| Cobra       | D3: 4:00 PM   | 34.3      | 27.27     | 14.68     | 15.13       |
| OneBetta    | D1: 9:00 AM   | 77        | 7.36      | 40.6      | 41.65       |
| OneBetta    | D2: 1:00 PM   | 53        | 17        | 80        | 50.00       |
| OneBetta    | D3: 4:00 PM   | 120       | 51        | 71        | 80.67       |

$^a$D1, D2, and D3 represent days 1, 2, and 3. On each day, canines were evaluated at three different times per day for the time taken to locate three training aids ($T_1$–$T_3$).

$^b$In each case, healthy avocado wood was used as a negative control and no alerts were observed.

### Table 2. The results of a 2-week trial with the laurel wilt pathogen cultures and negative control training aid materials for two canines, Cobra and OneBetta. This trial evaluated the ability of the canines to discriminate nontarget odors from the target odor. Each training aid type was stored in controlled odor permeations systems (COMPS). Treatments and corresponding responses by each canine (Alert/No Alert) are shown.

| Day | Canine name | Pathogen + malt agar$^a$ | Empty COMPS$^b$ | Empty COMPS | Empty COMPS | Malt agar only$^c$ |
|-----|-------------|--------------------------|-----------------|-------------|-------------|-------------------|
| 1   | Cobra       | Alert                    | No Alert        | No Alert    | No Alert    | No Alert          |
| 1   | OneBetta    | Alert                    | No Alert        | No Alert    | No Alert    | Alert             |
| 2   | Cobra       | Alert                    | No Alert        | No Alert    | No Alert    | No Alert          |
| 2   | OneBetta    | Alert                    | No Alert        | No Alert    | No Alert    | No Alert          |
| 3   | Cobra       | Alert                    | No Alert        | No Alert    | No Alert    | No Alert          |
| 3   | OneBetta    | Alert                    | No Alert        | No Alert    | No Alert    | No Alert          |

$^a$COMPS with the pathogen grown on malt agar.

$^b$COMPS bag without any pathogen content.

$^c$Pure malt agar sealed in a COMP bag with no microbial growth.
canines to pinpoint targets (Savidge et al., 2011). Canine performance was unaffected by climate conditions in a previous study with desert tortoises (Nussear et al., 2008) whereas, with respect to brown tree snakes in Guam, increasing humidity had a positive effect with performance, and lower wind speeds increased the success (Savidge et al., 2011).

Last, the canines after being presented with the laurel wilt pathogen, grown in pure culture, successfully demonstrated the ability to detect and alert to only the fungus (Table 2). The fact that the canines were able to alert to and detect the pathogen in laboratory-grown cultures, when they were primarily trained on the entire scent picture of the pathogen growing within avocado wood, is a testament to their specificity. This is not surprising as previous studies demonstrated this ability with canines trained on toxic mold species growing on wood (Griffith et al., 2007). The study on mold aimed to determine whether through the use of analytical chemistry, they could identify volatile signature differences between closely related species of mold and whether detector dogs could distinguish between nontoxic and toxic species. Whereas canines demonstrated >90% accuracy on toxic mold growing on wood, this translated to ~70% with laboratory-grown cultures (Griffith et al., 2007). This study also discussed the usefulness of analytical techniques to identify unique chemical signatures to improve training through more specific odor mimics as aids, and has also been investigated with explosives and drugs (Harper et al., 2005). It is also important to note that closely related species of fungi exist to the pathogen used in this study and it is unclear whether the canines would alert to potentially similar odors. The use of additional cultures of different species including those isolated from avocado trees is underway and will be evaluated in future studies.

Another interesting study by Wagggoner et al. (1998), discussed the effect of extraneous odors on the ability of canines to detect target odors. This study involved the masking of the target with increasing concentrations of nontarget odors (Wagggoner et al., 1998). This is significant as most training takes place in controlled environments indoors with no extraneous odors. The results demonstrated a remarkable ability to alert correctly to the target in the presence of masking odors, but that in extremely high nontarget odor environments, their detection capabilities can be reduced (Wagggoner et al., 1998). In the present study, the method of training in outdoor agricultural settings was well thought out to enable the canines to be accustomed to as many possible interfering odors as possible as they would experience them in their intended deployment application. This study demonstrated successful training, usefulness, and evaluation of the detection capabilities of scent-discriminating canines and provides a new tool in the arsenal in defense against plant diseases in an agricultural or outdoor, uncontrolled environment.

Conclusions
This study demonstrated the ability of scent-discriminating canines to locate laurel wilt–diseased avocado wood with high accuracy and speed. During the course of the study, 229 trials were performed, and only 12 false alerts were observed and 12 failures to alert. It was observed that canines are capable of high levels of performance even in harsh weather conditions such as high heat and humidity.

This study provided the proof of principle that canines can detect agricultural diseases such as laurel wilt and can be a powerful management tool if the disease is caught in its earliest stages. Laurel wilt disease is especially difficult and costly to manage. Once symptoms of this disease become visible to humans, it is already too late for treatment and that the tree must be removed as it is highly likely that the pathogen has spread to adjacent trees, through root grafts. Analagous to early cancer detection in humans (Godfrey, 2014; McCulloch et al., 2006; Moser and McCulloch, 2010), the ability to detect trees at an earlier disease stage would provide a significant advantage in the battle to stop the spread of this deadly crop disease.

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