Fire Ant-Detecting Canines: A Complementary Method in Detecting Red Imported Fire Ants

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Fire Ant-Detecting Canines: A Complementary Method in Detecting Red Imported Fire Ants

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ABSTRACT In this investigation, detection dogs are trained and used in identifying red imported fire ants, *Solenopsis invicta* Buren, and their nests. The methodology could assist in reducing the frequency and scope of chemical treatments for red imported fire ant management and thus reduce labor costs and chemical use as well as improve control and quarantine efficiency. Three dogs previously trained for customs quarantine were retrained to detect the scents of red imported fire ants. After passing tests involving different numbers of live red imported fire ants and three other ant species—*Crematogaster rogenhoferi* Mayr, *Paratrechina longicornis* Latreille, and *Pheidole megacephala* F.—placed in containers, a joint field survey for red imported fire ant nests by detection dogs and bait traps was conducted to demonstrate their use as a supplement to conventional detection methods. The most significant findings in this report are 1) with 10 or more red imported fire ants in scent containers, the dogs had >98% chance in tracing the red imported fire ant. Upon the introduction of other ant species, the dogs still achieved on average, a 93% correct red imported fire ant indication rate. Moreover, the dogs demonstrated great competence in pinpointing emerging and smaller red imported fire ant nests in red imported fire ant-infested areas that had been previously confirmed by bait trap stations. 2) Along with the bait trap method, we also discovered that ≈90% of red imported fire ants foraged within a distance of 14 m away from their nests. The results prove detection dogs to be most effective for red imported fire ant control in areas that have been previously treated with pesticides and therefore containing a low density of remaining red imported fire ant nests. Furthermore, as a complement to other red imported fire ant monitoring methods, this strategy will significantly increase the efficacy of red imported fire ant control in cases of individual mount treatment.

KEY WORDS red imported fire ant, bait trap station, detection dog, odor recognition, fire ant detection

The red imported fire ant, *Solenopsis invicta* Buren, an exotic species that originated from South America, was first discovered in the United States in the 1920s. Their spread to pan-Pacific countries such as Australia, New Zealand, Taiwan, and China took place around the early 21st century (Callcott and Collins 1996, ISSG 2010). Morrison et al. (2004) also predicted, based on their dynamic ecophysiological model of colony growth, the path of the red imported fire ant's future invasions to parts of Asia, Africa, southern Europe, and even to the Pacific island nations.

This notorious pest is capable of consuming a large quantity of food during its rapid propagation, devastating agricultural products, threatening the well-being of both livestock and humans, and seriously affecting the native animal species in the invaded areas (Adams 1986, Lofgren 1986, Allen et al. 1994).

The application of ant baits containing toxic chemicals or insect growth regulators (IGRs) over the infested areas is one of the most widely adopted strategies in red imported fire ant management. Dust, granule, or liquid insecticide intended for individual mount treatments also provide a quicker option for small-scale pest control. The red imported fire ant control support services in the United States have promoted a methodology combining broad-spectrum and single-nest treatment, known as the "two-step method." The two-step method is presently considered as the most cost-effective and environmentally friendly approach for highly red imported fire ant-infested areas (Drees et al. 1998, 2000). However, Drees reported that the overuse of the red imported fire ant pesticide "diazinon" produced massive amounts of waste and water pollutants that failed to

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pass the state and federal discharge standards in the United States (Drees 2003). Moreover, related research revealed that contaminated drainage ditches from the long-term insecticide applications might alter the water quality of streams and rivers and affect the aquatic communities (Heckman 1981, Liess and Schulz 1999). Therefore, an accurate assessment of the level of red imported fire ant infestation is needed before more precise insecticide treatments can be applied as to reduce both the environmental and economical costs associated with chemical control.

In monitoring red imported fire ant, visual inspection, pitfall traps, and bait traps are the most common methods adopted in detecting the red imported fire ant and determining the amount of treatment. For large-scale investigations, satellite imagery, aerial imagery, or both are used to provide areawide images of large red imported fire ant mound distributions (Vogt 2004a,b). Yet, the nests are usually broken and lack the obvious dune structures on sites where multiple red imported fire ant treatments have already been applied. Many of these areas consist of those that are less tolerant to fire ant infestation, i.e., places with significant human activities such as playgrounds, parks, hospital, power facilities, and nurseries. Once treated with pesticides and toxic traps, small amounts of nests may still remain, whereas newer nests also can emerge after treatment. On occasions, nests also are found to be concealed in grass sods or outdoor electronic appliances. Such situations render the visual inspection of red imported fire ant tedious and rather useless. Bait traps consisting of a food attractant inside a container are not capable of locating the actual site of the mounds because they are usually found by foragers; instead, they merely show that red imported fire ant exists somewhere within the vicinity of the baits. Thus, individual mound treatment cannot be applied efficiently and accurately to attain rapid red imported fire ant control in such low nest-density areas. Thus, the development of a practical method to rapidly locate red imported fire ant colonies in previously treated areas is necessary as to avoid costs associated with inaccurate and ineffective pesticide reapplications.

Animals rely heavily upon their delicate senses for survival, such is the case with the dog's olfactory system. Taking advantage of this trait, detection dogs are often trained to search for objects or creatures such as fruit, animal products, snakes, gypsy moths, termites, bed bugs, and other organisms by tracking their unique odors (Wallner and Ellis 1976, Welch 1990, Engeman et al. 1998, Brooks et al. 2003, Pfiester et al. 2008). Dogs have the great capability of accurately locating concealed targets with their nose even when it is wrapped in packages or enclosed in other materials (Brooks et al. 2003). We had demonstrated previously that dogs were able to pick up the scents of red imported fire ants (Huang et al. 2007). In Australia, off leash Labrador retrievers also were trained to freely search for red imported fire ant nests in the field with satisfactory results (Corcoran and McNicol 2009).

In this report, three beagles were successfully trained on leash to detect live red imported fire ants of varying quantity; discriminate them from other ant species; and perform a detailed field investigation of emerging and small red imported fire ant nests in a pretreated outdoor area, which provided the groundwork needed for the posttreatment of these individual mounts.

Materials and Methods

Red Imported Fire Ants. Red imported fire ants for dog training were collected from mounds in Ching-Pu, Taoyuan County, Taiwan. This was done by placing pieces of tissue paper upon the disturbed mounds, thereby allowing the collection of the ants that aggressively swarmed the paper. The red imported fire ant populated paper was then transferred into plastic zip-lock bags where the ants were subsequently anesthetized with CO_2. The paralyzed ants were then placed into a 50-ml capped tube with a square sieved opening (1.7 by 1.7 cm) allowing the scent to permeate out for dog training and testing.

Canines. Three neutered male beagles aged 6, 4, and 2 yr old were recruited in this investigation. They will be referred to as dog A, B, and C, respectively. All three dogs had previously received quarantine training to detect agricultural products in airport customs. The dogs were then further trained for red imported fire ant detection and used in subsequent experiments. In the red imported fire ant training program, the dogs were taught to inform their dog handler of red imported fire ant presence by sitting in front of the discovered targets (passive response).

Indoor Training for Red Imported Fire Ant Odor Recognition and Identification. Red imported fire ant odor recognition training was initiated indoors as a precursor exercise for 2 wk. The purpose of the indoor training was to avoid the influence of weather and other extrinsic odors commonly encountered outdoors. Considering quarantine issues and sample availability, only dead red imported fire ants were allowed in the indoor training. The indoor training consisted of two steps: 1) ≈50% of a set of tubes described previously were filled with ≈100 previously frozen red imported fire ants. Each set was then hidden in a metal can fixed on a wooden board. The handler would lead the dog to sniff the cans that contained the dead red imported fire ants and verbally encourage it to sit by the cans; a food reward of two dog biscuit pellets was given for correct responses. Simultaneously, the dogs also were taught not to react to or sit by empty vials through oral instructions. 2) To prevent dogs from reacting to odors from non-red imported fire ants, the dogs were further educated to refrain responding to tubes with ≈100 frozen laboratory reared *Solenopsis germinata* F., *Anoplolepis longipes* Jerdon, *Polyrhachis dives* Smith, *Crematogaster rogenhoferi* Mayr, or *Tetramorium* sp. 1. Before proceeding to the outdoor training, the dogs are presented with sets of 30 tubes each containing one to five tubes of dead red imported fire ants, they must consistently recognize at least 20 tubes of red imported fire ants in total from all the sets combined. In this case, the number of sets of 30 tubes presented can vary, but as long as the dog can successfully identify 20 tubes in total of red imported fire ants in the duration of the tests, they are allowed to go onto the next training exercise.

Outdoor Training for Red Imported Fire Ant Odor Recognition and Identification. A set of tubes with 100, 50, 10, or no live red imported fire ants were buried in a lawn with the lids revealed above the ground. Through the same methodology described previously, the dogs were guided to detect the live red imported fire ant-containing tubes and to ignore the empty tubes. To discriminate red imported fire ant odors from non-red imported fire ant odors, 100 live laboratory raised *S. germinata, A. longipes, P. dives, C. rogenhoferi,* or *Tetramorium* sp. 1 were collected in tubes and arranged randomly during the training. Dogs were taught to only recognize red imported fire ants and to discriminate them from the non-red imported fire ant species as in the indoor training.

Field Training for Red Imported Fire Ant Nest Detection. Initially, red imported fire ant nests varying in size were identified in the field by visual inspection. As a warm up, each dog must accomplish two rounds of outdoor live red imported fire ant training, as described above, for purposes of recalling their memories about the scents of red imported fire ants. After recollection of their red imported fire ant odor memories, the dogs were guided to the general area to sniff out red imported fire ant nests and to sit by the targets. Piles of rocks, soil mounds, or nests of non-red imported fire ant species were selected as negative controls during the training. The dogs' false indications of non-red imported fire ant targets were discouraged verbally by their handlers. To avoid being attacked by red imported fire ants, dogs also were trained to sit away from detected nests right after sniffing.

Each dog received 2 h of outdoor training in addition to 2 h of field training each day: one session in the morning and the other session in the afternoon. The whole process was completed within 2 wk. Alternate training sessions prevented dogs from being bored and distracted from the work, which could lead to lower efficiency during training. Field training also provided more complicated situations that sharpened their capabilities to pick up more unpredictable scents of red imported fire ants. Before proceeding to the assays of the dogs' ability in locating red imported fire ants, they must consistently recognizing a combined total of 10 tubes of red imported fire ants out of sets of tests consisting of 30 tubes in an outdoor setting, just as they did before proceeding to the outdoor training.

Olfactory Detection of Varying Numbers of Red Imported Fire Ants. Fifteen tubes of red imported fire ant were prepared as targets, in which three sets of five tubes each contained 100, 50, and 10 live red imported fire ants. A further 45 empty tubes were used as nontargets. Tubes were randomly selected and buried in the ground in a line, 3 m away from one another with their lids exposed to the air. Each dog was assayed with the 60 (15 red imported fire ants + 45 empty) tubes once per day, for a period of 10 d. There were, in total, 50 chances for each of the 10, 50, and 100 red imported fire ant tubes to be recognized by each dog. "Positive indication" was defined as a dog's correct response of sitting by the target red imported fire ant tubes. The dogs' positive indication rates in discerning 100, 50, or 10 ant samples were compared with each other by the Mann–Whitney *U* test by using the online statistical analysis package VassarStats as provided by Vassar College (Lowry 2010).

Ability to Differentiate Red Imported Fire Ants From Other Ant Species. To determine whether the dogs would be confused by unfamiliar ant scents that did not occur in previous training sections, and to provide them with more chances to recognize other non-red imported fire ant scents, four sets of 12 metal cans, each containing 10 field-collected *C. rogenhoferi, P. longicornis, P. megacephala,* or red imported fire ants were prepared as targets (red imported fire ant) or nontargets (non-red imported fire ant species) as described above. The metal cans were randomly selected and arranged in a line three meters away from one another. Each dog's distinction ability was assayed with the 48 cans once per day for a period of 10 d. There were, in total, 120 chances for the red imported fire ant tubes to be detected by each dog. "Indication" was defined as a dog sitting in front of a can. The Indication rates of the four species were compared with each other by the Mann–Whitney *U* test.

Searching for Emerging and Residual Red Imported Fire Ant Nests in the Field Through Detection Dogs Complemented by the Bait Trap Method. The investigated site is a red imported fire ant-infested grassland of 6,160 m^2 located at the Taoyuan County stadium in Taiwan; the stadium had been repeatedly treated with pesticides and IGR baits for nearly 2 yr. The ground in the area were without noticeable nest mounds but contained previously surviving nests and new emerging nests red imported fire ant nests that are hard to detect merely by eye. This grassland is thus selected for a survey of residual and emerging red imported fire ant by detection dogs with the additional help of bait trap data.

The grassland was equally divided into seven smaller subareas, each $\approx$880 m^2. First, for bait traps, $\approx$220 uncapped tubes containing potato chips were distributed equally to each subarea (with 1,540 tubes in total in the whole testing ground). After 2 h, the tubes were collected and capped for further species identification. The number and locations of the tubes were then recorded.

Second, the dog handler led the dogs to search for red imported fire ant in the same area immediately after the completion and removal of the baits traps. Dogs A and B were selected for the detection process. Places containing $>$10 red imported fire ants were defined as an ant nest. Sites of detected red imported fire ant nests (as opposed to forage tunnels) were further verified by shovel excavation or potato chip bating, and the number of nests was recorded and mapped on the same plot mentioned above.

The whole procedure ended in 7 d, with the 6,160-m^2 area fully investigated, resulting in a complete map of the red imported fire ant distribution as

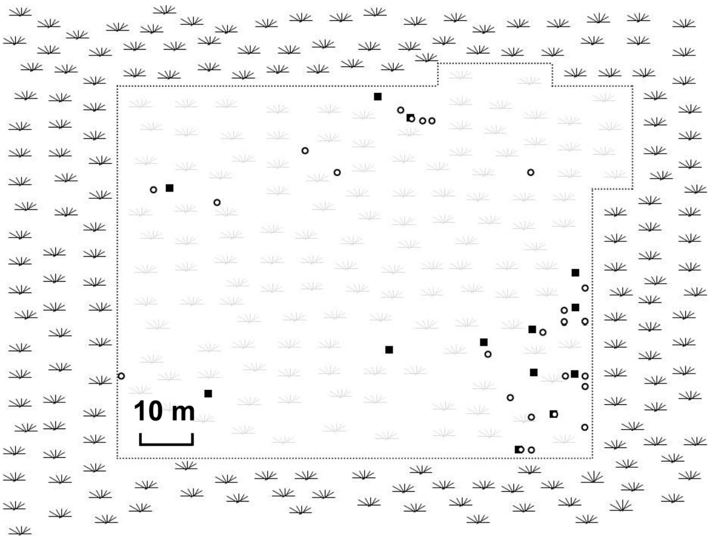

Fig. 1. Distribution of bait trapped red imported fire ants (open circles) and dog-detected red imported fire ant nests (closed squares) on the site of interest. The dotted line enclosed region illustrates the area that was searched using the detection dog–bait trap method.

revealed by the bait trap data combined with the nest detection data from the dogs (Fig. 1).

For data analysis, a grid with cells of 2 by 2 m was superimposed onto the map of the surveyed area to measure distances between the bait trapped red imported fire ant and their nest sites. Concentric circles centered at red imported fire ant nests with various radiuses from 0 to 18 m were plotted at increments of 2 m. The larger the plotted circle area, the more trapped red imported fire ants were included within its radius. The percentage of trapped red imported fire ants in each radius was then calculated from the number of trapped red imported fire ants in each extending radius over the total number of trapped red imported fire ants in the entire survey area. A logarithmic regression curve of the accumulation percentage of red imported fire ants versus radii was generated to show their correlation.

Results

Dogs' Olfactory Ability in Discerning Various Quantities of Red Imported Fire Ants. The experimental data were separated into two parts and analyzed as follows: one part was the detection dogs' reaction toward the tubes which contained 10, 50, or 100 live red imported fire ants. Positive indications were defined as when the dogs successfully located the tubes containing red imported fire ants. All three trained detection dogs were capable of locating the red imported fire ant tubes, with an overall positive indication rate of >98% (Fig. 2). There were no significant difference between dogs and their capabilities of detecting 10, 50, or 100 ants ($P < 0.05$; Mann–Whitney U test). The other part was the results of the detection dogs' responses to empty tubes. False positive indication is defined as when the dogs reacted to empty tubes. In this category, the false positive indication rate was <2% for each dog. We also observed a lack of significant difference among the dogs' abilities in detecting red imported fire ants. Overall, the results indicate that all three trained detection dogs were able to detect red imported fire ant populations of >10 ants. This threshold is preferable for locating grouped red imported fire ants or red imported fire ant nests rather than solo foragers.

Assessment of the Dog's Ability to Distinguish Red Imported Fire Ant From the Other Species. Four ant species, *C. rogenhoferi*, *P. longicornis*, *P. megacephala*, and red imported fire ant, were prepared and tested. Ten ants from each species were presented alive to the detection dogs as described in Materials and Methods. The general correct indication rate on red imported fire ant by the dogs was ≈93%. This result is signifi-

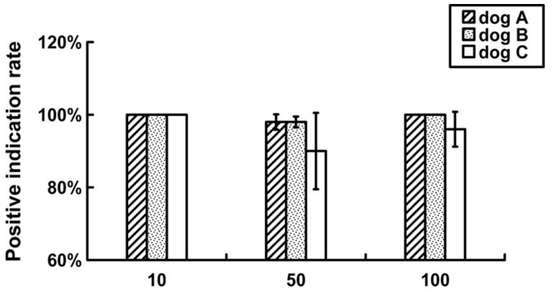

Fig. 2. Positive indication rates of detection dogs for red imported red ants (RIFA) in tubes containing 10, 50, and 100 ants. Positive indication rate is the mean percentage of positive indications over total indications by dogs. The result shows no significant differences among the dogs' capability in discriminating the varying quantities of ants ($P < 0.05$; Mann–Whitney U test).

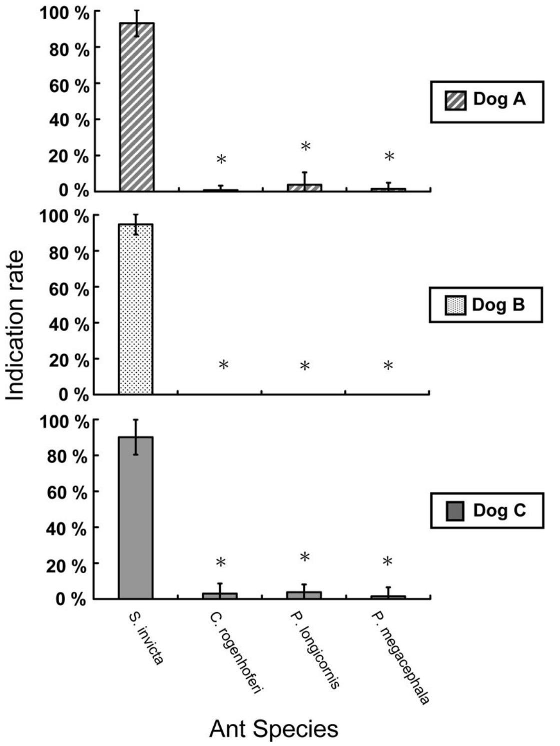

Fig. 3. Percentage of indication rates for the four ant species by detection dogs. Indication rate is the number of dog indicated tubes over the total tube numbers. Columns represent the mean ± SD of 10 repetitive tests. The asterisks indicate the significant difference between the non-red imported fire ant species and red imported fire ants ($P > 0.05$; Mann–Whitney U test).

cantly higher ($P > 0.05$; Mann–Whitney U test) than the 4% from the dogs' response to other species (Fig. 3). The results revealed that, after training, the three detection dogs had all acquired the capability to correctly differentiate red imported fire ants from the other local species of ants.

Detection Dog and Bait Trap Joint Field Survey. Twenty-five tubes of trapped red imported fire ants were captured by bait traps and 13 nests were located by the dogs (Fig. 1). Besides this, we also found tubes occupied by other non-red imported fire ant ants, with numbers from a few to hundreds depending on the tubes' location and the geological distribution of the different species of ants. The total number of tubes with non-red imported fire ants was estimated to be ≈30% out of the 1,540 tubes.

According to the logarithmic regression curve ($R^2 = 0.976$) showing the percentage of accumulated bait trapped red imported fire ant in the circled area versus the radii, 60% of captured red imported fire ants were located within a 2-m radius centered at the closest nest. As the radius extended to ≈14 m, almost 90% of captured red imported fire ant were included in the circle. Thus, only ≈10% of the red imported fire ants wandered to regions outside of the radius of >14 m of the circle centered at the nearest nest (Fig. 4).

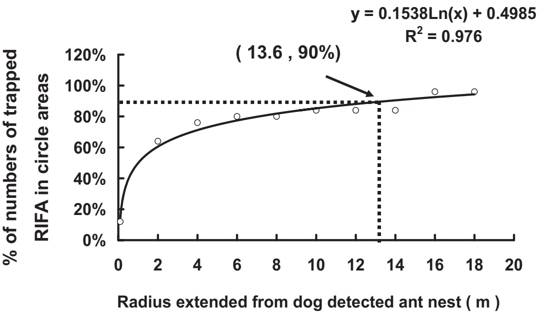

Fig. 4. Relationship between the dog-detected ant nests and the bait-trapped red imported fire ants (RIFA). Open circles, percentage of trapped RIFA located in different radii extended from the nests. An arrow indicates that 90% of trapped RIFA were located within ≈14 m from the nests.

From the experimental results, it was concluded that most of the red imported fire ants (≈ 90%) were foraging within 14 m from the nests and that only ≈10% red imported fire ants foraged at distances further than 14 m. The longest observed distance covered by ants foraging from the nearest nest was ≈26 m.

Discussion

All three trained dogs demonstrated high positive indication rates (≈98%) in detecting red imported fire ants. Their false positive rates on the empty tubes were <2%. The indication rate on *C. rogenhoferi*, *P. longicornis*, and *P. megacephala* were also <4%. All these results not only suggest the great capability of dogs for locating red imported fire ant but also met the required minimum acceptable detection threshold among other insect detection dogs as suggested by Brooks et al. (2003). In his report, the termite detection dogs had an accuracy of 95.93% in detecting eastern subterranean termites, *Reticulitermes flavipes* (Kollar). Bed bug-detecting dogs showed an accuracy of 97.5% in locating live bed bugs as well as a 90.0% accuracy in locating viable bed bug eggs (Pfiester et al. 2008). A trained German wirehaired pointer could search for screwworms, *Cochliomyia hominivorax* (Coquerel), with 99% positive indication rate, and three German shepherds were able to sniff out the gypsy moth, *Lymantria dispar* (L.), egg masses with an accuracy of 95% (Welch 1990, Wallner and Ellis 1976).

Many factors may affect the detection accuracy of the dogs, ranging from the training protocols, equipment, abiotic factors such as temperature and wind, and the maintenance of the dogs' memories toward specific odors. The handler's interpretation of the dog's behavior as well as the length of dog's detection time also contributed to their effectiveness (Wallner and Ellis 1976, Welch 1990). Although all three dogs received the same training process and maintenance and were tested under identical conditions, dog C's positive indication rates on red imported fire ant fluctuated more than the other two dogs. Dog C was observed to become easily excited whenever unfamiliar dogs other than dogs A and B were present, especially in the outdoor environment. Such behavior often distracted it from active searching and affected the accuracy of detection. Behavioral corrections were attempted but were unsuccessful. Considering this, only dogs A and B were selected for the field test of investigating the red imported fire ant nests at the Taoyuan stadium.

The Australian canines were taken off their leashes before searching and released to perform free search, once the nest is found the dogs wagged their tails excitedly at the site and were subsequently rewarded with playtime consisting of catch and retrieve. Our dogs however were trained to search for fire ants while being guided on leash by a handler; this gives the handler more control over the specific search area and the search speed. It also prevents the dogs from getting too close to the danger of fire ant swarms and if necessary, their mouths, noses, and paws can be inspected for red imported fire ant immediately. Furthermore, a food reward was given instead of playtime after each successful detection, which ensures that our dogs are well nourished and not too tired or overexcited from playing. Every dog was capable of working for 4 h/d, dogs that worked over this time frame were less motivated and easily distracted, hence leading to lower detection rates.

Our field investigation suggests that trapped red imported fire ant were not always adjacent to their respective nests. Such phenomena could possibly lead to an over estimation of the red imported fire ant distribution if the investigation was conducted merely by the bait trap method. Therefore, in practice, excessive pesticides intended for individual mound treatments could have been applied and unnecessary labor costs would then incur as a result especially in areas of low nest density. Oi et al. (2004) observed that when red imported fire ant nest densities are low (≦15 nests per ha), <30% of red imported fire ant nests can be detected by traps with attractants. Low efficacy of bait traps implies that further improvements are needed in detecting nests at low density locations. Compared with the bait traps, the detection dog has an overall positive indication rate of >98% based on the assay for olfactory detection of the various number of red imported fire ant, as shown in Fig. 2. Hence, they are ideal for narrowing down the rough estimations provided by the bait traps and would allow for more accurate determinations of the specific locations of the individual nests. Once more precise nest site information has been obtained, the unnecessary costs associated with overestimations of nests sites and over applications of treatments can be avoided, allowing on site or regional countermeasures to be applied more efficiently at the individual mound level as opposed to inadequate area wide treatments.

Besides inspecting red imported fire ants in the soil, red imported fire ant detection dogs are also readily deployable to greenhouses, farms, container distribution centers, or ports-of-entry to examine the incoming or outgoing products for any possible red imported fire ant infestation. Its wide array of applications has the potential to improve the accuracy and reduce the costs of red imported fire ant quarantine programs around the world.

This study was conducted to assess the trained dogs' capabilities in detecting live red imported fire ants as well as their nest in the field. The above-mentioned results indicate that dogs can be trained to detect red imported fire ants, discriminate it from three other species, and locate red imported fire ant nests in the field. Of course, this methodology is by no means a replacement to the conventional detection methods; instead, the application of the red imported fire ant detection dogs is meant to act as a supplement to the other red imported fire ant inspection methods by providing more accurate identifications of hidden nests.

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