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Pilot Study for the Use of Scent-Detection Dogs in Surveys for the Endangered Pima Pineapple Cactus (*Coryphantha scheeri* var. *robustispina*)

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June 2017

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Section 1. Introduction

1.1 Study Purpose and Background

H. T. Harvey & Associates conducted a study to assess the efficacy of using ecological scent-detection dogs to survey for the cryptic Pima pineapple cactus (*Coryphantha scheeri* var. *robustispina*) (PPC) (Photo 1). To achieve the overall research goal, two scent-detection dogs were trained to recognize the scent of PPC and to discriminate the scent from that of sympatric cactus species. A trial phase was conducted with two detection dog teams, each consisting of one detection dog and one handler, to quantify the accuracy, sensitivity, and specificity in finding PPC.

Photo 1. Pima Pineapple Cactus Training Specimens

PPC is listed as an endangered species by the U.S. Fish and Wildlife Service (USFWS) and as a highly safeguarded species by the State of Arizona. It grows on gently sloping alluvial fans, ridgetops, and basins of the Sonoran Desert scrub and semidesert grasslands of southern Arizona and northern Mexico (USFWS 1993). This cactus has proved difficult to detect during traditional transect surveys because it is sparsely distributed within its range and habitat and is small and often camouflaged by tall grasses or other vegetation. Recommended protocol surveys for PPC are intensive and time consuming, requiring a three-tier approach comprising short-distance repeated transect surveys, local-area searches centered around PPC individuals detected during the survey, and focused ground-level surveys for seedlings, conducted in a 50-square-meter area around randomly sampled mature plants (Roller 1996). This intensive protocol was developed in response to empirical evidence gained during previous surveys. For example, Roller (1996) describes one PPC survey in which researchers conducted repeated transect surveys and found 100% as many plants on the second and third passes as on the first pass and did not find all the PPC in the plot until the sixth survey was complete.

The excellent olfactory abilities of scent-detection dogs offer an alternative or complementary survey method that may improve detection of PPC; lead to a better understanding of the species' distribution, abundance, and demography; and efficiently provide a basis for answering outstanding research questions. Dogs are able to distinguish particular scents from among a vast amount of scent information in their environment and, through proper training, can locate a specific odor, discriminating among all other environmental odors. H. T. Harvey & Associates deploys ecological scent-detection dogs for surveys of special-status or invasive species, and avian and bat fatalities. Suitable dogs are carefully selected and the trainers and handlers spend numerous hours training them to efficiently search for the scents of target species and to alert their handlers to the source of those scents.

The limitations associated with human-only surveys for cryptic species, such as PPC, may be reduced by incorporating trained scent-detection dogs. Dogs have been used with great success to assist in finding several species of plants (Vesely 2008, Goodwin et al. 2010, U.S. Customs and Border Protection 2016). Human-only visual surveys for rare plant species often are biased toward mature and flowering life stages (Palmer 1987), but dogs have been recorded detecting odor originating from a target plant's roots (Goodwin et al. 2010), as well as from plants that are dead and desiccated (Vesely 2008). Judging from the work done to date, using scent-detection dogs is likely to improve the efficiency of survey efforts for PPC, increase the number of PPC detections, and provide a less costly and more reliable alternative to traditional sampling for this species. To leverage this potential advantage, we conducted a study with the following objectives:

- Demonstrate proof of concept for detection dog surveys of PPC.
- Quantify the accuracy, sensitivity, and specificity in detecting PPC for two detection dog teams.
- Ultimately improve the probability of PPC detection and the efficiency of surveys.

Section 2. Methods

2.1 Overview

Two dog-handler teams identified for the study underwent three stages of training. The first stage was scent recognition container drills, the second was scent discrimination, and the third was scent recognition field training. To advance to the second and third stages of training, each detection dog-handler team was required to pass a test to assess readiness for more advanced levels of training. When all training was completed, the detection dog-handler teams participated in a field trial, where they were tested for accuracy, sensitivity, and specificity to PPC odor.

2.2 Training Samples

Potted PPC plants were used to train the detection dogs. Using potted plants enabled us to manipulate the location of the target scent in controlled training exercises and trials. The PPC plants were transported from the Arizona-Senora Desert Museum (ASDM), located in Tucson, Arizona, to H. T. Harvey & Associates' detection dog training site in Acton, California. We obtained a copy of ASDM's recovery permit for native species federally listed as endangered or threatened (TE022190-0), as well as a letter from USFWS granting permission for the transfer of the plants. These two documents remained with the plants during transport and storage.

2.3 Scent Recognition

The study took place in Los Angeles County, California, in an arid climate (Mojave Desert) that mimicked survey conditions but where other threatened and endangered species would not be negatively affected. The testing was conducted outside the range of these species to maintain control of the location and number of targets in the study area.

Photo 2. Scent Recognition Container Drill with Kaia

During the scent recognition stage, container drills were used to teach the dogs to associate the target odor with a play or food reward. To begin, each dog was led along a row of ventilated 5-gallon buckets (Photo 2); one contained a potted PPC plant (Photo 3), and the others were empty or contained controls composed of additional

Photo 3. PPC in Containers Used for Scent Recognition Training

materials used during training, such as pots and soil identical to those used for the PPC plants. When the dog sniffed the container with the target odor, the dog was rewarded immediately and enthusiastically with its favorite toy or food. This exercise was repeated until the dog associated the target scent with a positive event, as indicated by the dog's display of anticipatory behavior when it smelled the target odor. Anticipatory or "alert" behavior varies among dogs but often includes a sudden change in direction of movement or a change in posture, combined with focused attention toward the handler in anticipation of the reward. This behavior demonstrates to the handler that the dog associates the target odor with the reward. As the training advanced, the number, position, and types of containers varied. Introducing multiple variables teaches the dog that it cannot rely on a pattern to find the scent and that the location of the scent is always random. Enclosing the target scent in various types of containers teaches the dog to rely solely on its sense of smell, which is particularly important when training to find targets that are difficult to see.

After each dog appeared to consistently recognize the target odor, a scent recognition test was performed in which each dog-handler team was required to successfully indicate one target container randomly placed in a linear arrangement with nine control containers. The handlers were unaware of the placement of the target container. When the dog-handler teams successfully completed 10 consecutive scent recognition tests, the teams advanced to the scent discrimination phase of training and trial.

2.4 Scent Discrimination

During the scent discrimination training stage, the dogs were taught to distinguish between PPC scent and the scent of sympatric cactus species and other succulents. This clarification was achieved by introducing the dogs to nontarget scents and then eliminating them from the dog's perception of what constitutes the target scent through repetitive and selective reward delivery. Container drills were performed again, but instead of using only target containers and control containers, nontarget containers were added to the search. Golden barrel cactus (*Echinocactus grusonii)*, paddle cactus (*Opuntia* sp.), cholla cactus (*Cylindropuntia* sp.), and various ornamental succulents were used as nontargets (Photo 4). The barrel and paddle cacti were acquired from ASDM and are sympatric with PPC. Cholla samples were collected from private property in Los Angeles County, and the ornamental succulents were purchased from a nursery.

During the container drills, the handlers were aware of the location of the targets and nontargets to allow for appropriate reward delivery. The handler ignored the dog if it alerted to a nontarget or a control, but rewarded the dog immediately and enthusiastically if it performed an alert in response to the target. These drills were repeated numerous times until the dog had completed several sessions of searches without alerting to nontarget or control containers.

Photo 4. Nontarget Training Plants: (Left to Right) Golden Barrel Cactus, Ornamental Succulents, Cholla Cactus, and Paddle Cactus

When the lead trainer determined that the dog was successfully discriminating the target from the nontargets, the dog's readiness for the field trial phase was assessed by conducting a scent discrimination test. Ten identical containers were placed in a line. Each of the 10 containers held either a target scent, a nontarget scent, or a control. The number of targets (0–3), nontargets (1–4), and controls (1–3) and their placement in the linear arrangement was chosen using a random number generator. The individual PPC plant, the nontarget species, and the type of control also were randomly assigned for each search. Empty containers were placed in the remaining locations in the line. The handlers were not aware of the number or location of targets, nontargets, or controls.

The line of containers was searched by each dog-handler team in 10 consecutive scent discrimination tests. The placement of targets, nontargets, and controls changed with each test. The 10 consecutive tests were repeated until the dog-handler team correctly indicated at least 80% of the targets without falsely indicating a nontarget. To achieve this, the handler had to determine when the dog was displaying an alert behavior and when the dog had searched all available containers confidently enough to call the session complete. An observer with knowledge of the contents of each container recorded when the handler indicated a target and when the handler determined the session complete. When the handler announced that she believed the dog was alerting to a target, the observer said "yes" or "no" to the handler. This step was required to avoid rewarding the dog for indicating a nontarget. The handler was not made aware of any missed targets until all 10 tests were complete.

2.5 Field Scent Recognition

Photo 5. PPC Detection Dog Searching Tall Vegetation for Hidden PPC Target

During the field scent recognition test, the dog must find a target randomly placed on the landscape, in a defined area. This stage tests the dog's ability to generalize the initial training to a different context, one more closely resembling an actual search for the species in the field. Ten small plots (40 feet by 40 feet) were delineated, and one target was placed in each plot. The handlers were unaware of the placement of the targets. Because the targets are so large, they were camouflaged among tall grasses and vegetation so as to visually obscure the target from the handler and the dog (Photo 5). The teams were required to find the target in each of 10 plots during two rounds of five-plot searches. A total of 10 correct detections of the target was required to pass the field scent recognition test and move on to the field trial.

2.6 Field Trials

After the scent recognition (with containers and in a field setting) and scent discrimination training and testing stages were complete, each team performed six field trials to quantify the detection dog's accuracy, specificity, and sensitivity to the target scent in realistic survey conditions. Six 0.5-acre search areas were delineated in a high desert environment of Los Angeles County. Targets (0–5), nontargets (0–4), and controls (0–3) were placed in the search area, with the number and location of each randomly generated for each trial. The individual PPC plants, the species of nontarget, and the type of control placed were randomly assigned for each trial. All samples were again camouflaged so as not to visually cue the dog or handler (Photo 6).

Each field trial had a double-blind design. A field assistant placed the targets, nontargets, and controls at the randomly assigned coordinates, and a different individual recorded the data as each dog-handler team searched the plots. Both the handler and the data recorder were unaware of the number and locations of targets during each trial. The double-blind design was implemented to ensure that the data recorder did not unknowingly cue the dog to a target location.

The approach to the search is carefully determined by the highly skilled handlers and the effectiveness of various search patterns and pace are affected by the terrain, vegetation density, and weather characteristics, such as wind direction and speed and amount of relative humidity. Survey patterns can change each day or several times wtihin a day, depending on the conditions observed in the search area. Because detection dog searches are customized to suit these variables, they differ from traditional human-only transect surveys in that they do not necessarily follow straight lines or uniform spacing patterns. For example, it may be necessary for detection dog teams to obtain complete coverage of the plot by traversing an area outside the survey plot, allowing the wind to move the scent toward the dog.

Photo 6. Examples of PPC Sample Camouflage for Field Trial

Each detection dog exhibits a different search style and the unique traits of each dog dictate how the search is conducted. For example, some dogs are independent and cover large areas very quickly without much assistance from the handler while other dogs are more methodical and detail-oriented. The two dogs chosen to be trained on PPC are no exception—they require different approaches to the survey and different handling techniques. Bolt, a female cattle dog, works best off-leash and excels at detailing the search area with minimal direction from the handler. Bolt is, however, acutely aware of the handler and is responsive to direction and recall and was worked off-leash for all six survey plots. Kaia, a female shepherd mix, works best on-leash for small search areas. Kaia is excitable, especially at the beginning of searches, and the leash slows her down and encourages her to be more detailed in the search. After Kaia has searched for a few hours, she settles into the appropriate pace and can be taken off the leash; therefore, Kaia was worked on-leash for the first five plots she surveyed and off-leash for her last plot.

After all data were collected from the six trials, all true positives (dog alerts to target), false positives (dog alerts to nontarget), true negatives (dog ignores nontarget), and false negatives (dog misses or ignores target) were tallied. These four response types were analyzed using a confusion table (R package Caret) to calculate sensitivity, specificity, and accuracy for the PPC dog-handler team.

Section 3. Results

3.1 Scent Recognition

During scent recognition training, the dog was taught to associate the target odor with a play or food reward and was then tested on its ability to consistently recognize the target scent in preparation for discrimination training. Both dog-handler teams passed the scent recognition container test on the first attempt, on December 9, 2016, achieving a positive alert to the target container in each of 10 separate tests.

3.2 Scent Discrimination

The goal of the scent discrimination stage of training was to determine the readiness of the dog to graduate to the field scent recognition phase and ultimately the field trial phase, in which field performance is tested to ensure that the dog clearly exhibits an understanding of what constitutes the target scent and what does not. Success during the scent discrimination stage of training ensures greater efficiency during surveys, reducing the time and energy that may otherwise be spent investigating nontarget plants. In addition, during actual PPC surveys, the dog may recognize and alert to hard-to-identify PPC, such as shriveled or dead plants, sprouting plants, and even root systems. If there is high confidence that the dog can discriminate the scent of PPC, these cryptic or atypical specimens will not be overlooked.

To pass the scent discrimination test and advance to the field trial, the dog-handler teams were required to complete the test with at least 80% detection of targets and no alerts to nontargets (false positives). Each dog-handler team passed the scent discrimination test on January 24, 2017. Dog-handler Team 1 successfully identified 16 out of 20 targets (80%), and dog-handler Team 2 successfully identified 19 out of 20 targets (95%), with a combined 87.5% detection of targets and no false positives (Table 1).

Table 1. Scent Discrimination Results

Classification	Behavior	Team 1 Results	Team 2 Results
True positive: correct classification of a target	Dog alerts to a target, and the handler confirms the alert	80% success in classifying targets (16 out of 20)	95% success in classifying targets (19 out of 20)
False positive: false classification of a nontarget as a target	Dog alerts to a nontarget, and the handler characterizes it as an "alert"	0 false positives	0 false positives
True negative: correct classification of a nontarget	Dog-handler team misses, ignores, or passes the nontarget or control	100% success in classifying nontargets and controls	100% success in classifying nontargets and controls

3.3 Field Scent Recognition

After completion of the scent recognition and scent discrimination container drills and trials, the detection dog teams then advanced to the field scent recognition phase to test the dogs' ability to detect PPC in an environment more closely mimicking a field search scenario. Both dog-handler teams passed the scent recognition field test on the first attempt, on January 25, 2017. Both teams detected the target in each of 10 plots.

3.4 Field Trials

The dog-handler teams completed six field trials each on April 13 and 14, 2017. The random number–generated sample sizes yielded a total of 17 target PPC, 14 nontarget plants, and 11 controls placed in each team's combined search areas (Table 2). Figure 1 depicts the six survey areas and the results of the random number-generated samples sizes and locations of each sample (i.e. target, nontarget, or control). Each survey plot required approximately 30 to 45 minutes to survey. As described in the Methods, each handler chose a survey pattern best suited to each dog and each plot. Figure 2 depicts the survey of a plot as an example of the handlers' chosen search patterns. In this example, one can see that Handler 1 detected all the PPC targets (with some GIS error taken into account) and Handler 2 detected all the targets except the one farthest east. Some handler tracks intersect with nontargets and controls and indicate that the dog investigated the scent, decided it was not PPC, and moved on without an alert.

Dog-handler Team 1 detected 14 of 17 available targets and did not alert to any nontargets. Dog-handler Team 2 detected 16 of 17 targets and alerted to one nontarget (Table 3).

Table 2. Randomly Assigned Distribution of Each Sample Type by Plot

Plot Number	Number of Targets	Number of Nontargets	Number of Controls
1	1	2	0
2	4	2	1
3	5	2	3
4	5	5	5
5	2	2	0
6	0	1	2
Total	**17**	**14**	**11**

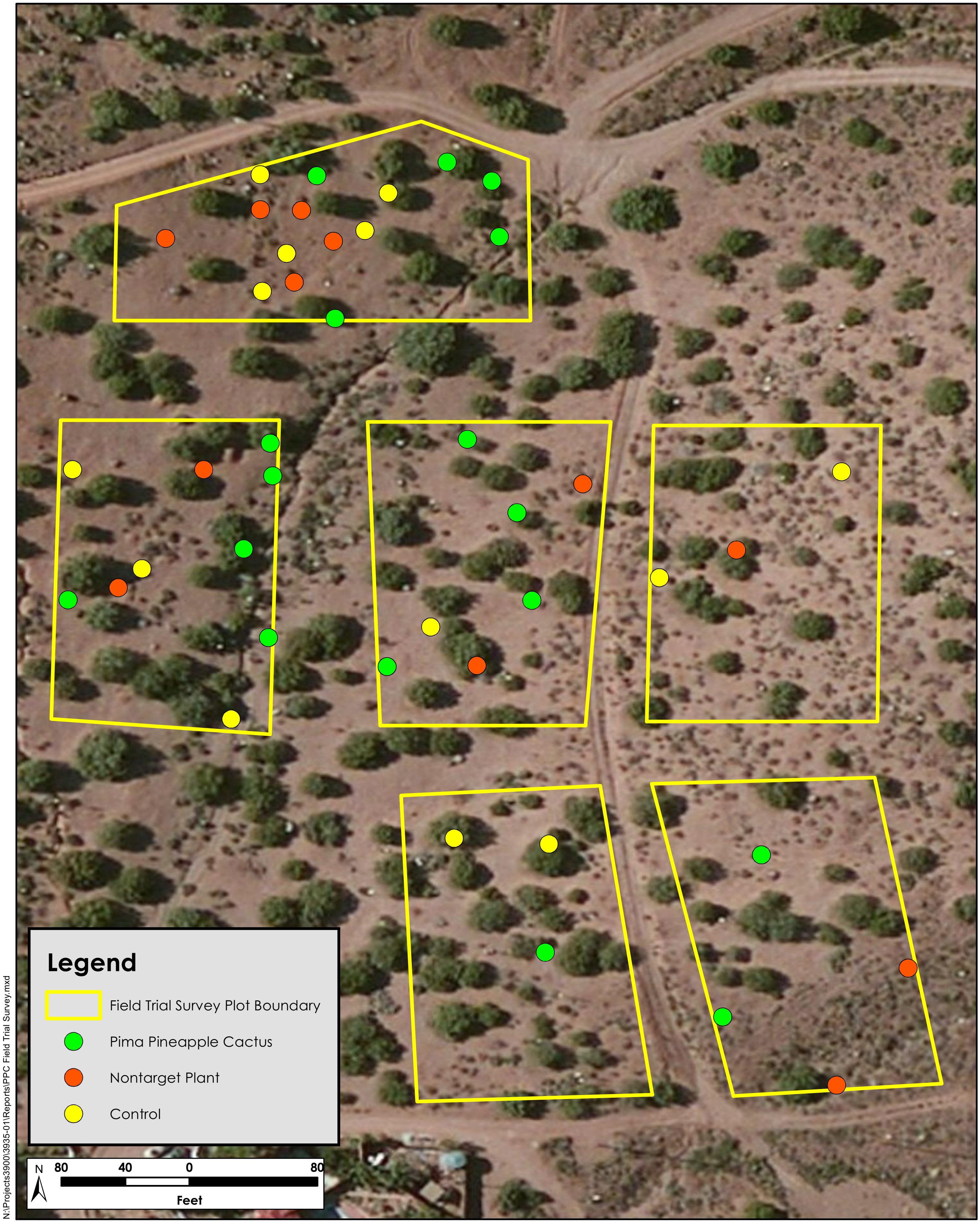

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Figure 1: Detection Dog Trials for Pima Pineapple Cactus

Pima Pineapple Cactus Report (3935-01)

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Figure 2: Example Handler Tracks for Detection Dog Trials
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Table 3. Field Trial Raw Data

Dog-Handler Team	Number of Trials	Number of Available Targets	Number of Targets Detected	Number of Available Nontargets and Controls	Number of Nontargets or Controls Detected
1	6	17	14	25	0
2	6	17	16	25	1
Total	**12**	**34**	**30**	**50**	**1**

Each team's trial results are reported separately and, to maximize sample size, combined. The overall *sensitivity* to the target scent is 0.88 (Table 4). *Sensitivity* is defined as the success rate of the dog performing an alert to all available targets and the handler recognizing the alerts. In other words, dog-handler Team 1 and 2 detected 82% and 94% of the 17 PPC, respectively, during six trials. The combined sensitivity for both teams for the 34 targets is 88%. The *specificity* of dog-handler Team 1 is 1.00 and dog-handler Team 2 is 0.96, with a combined sensitivity to the target scent of 0.98. *Specificity* is defined as the rate at which the dog correctly passes a nontarget scent. Team 2 confused the scent of golden barrel cactus with the scent of PPC once, and neither team confused the scent of paddle cactus, cholla cactus, or ornamental succulents with the scent of PPC. The overall *accuracy* of dog-handler Team 1 is estimated to be 0.92, with a confidence interval of 0.80 to 0.98. The accuracy of dog-handler Team 2 is estimated to be 0.95, with a confidence interval of 0.83 to 0.99. The combined accuracy of both teams is estimated to be 0.94, with a confidence interval of 0.87 to 0.98. In other words, when PPC and nontarget cacti and succulents are in a plot, the dog-handler teams correctly classify the target scents and nontarget scents 94% of the time.

Table 4. Field Trial Results

Classification	Behavior	Results
Sensitivity (true positive success rate)	Dog detects and alerts to a target, and the handler confirms the alert	88% success in detecting and classifying targets (30 out of 34)
Specificity (true negative success rate)	Dog-handler team misses, ignores, or passes a nontarget or control	98% success in classifying nontargets and controls
Accuracy (probability of correct classification)	Dog performs an alert to targets, and the alert correctly indicates targets; dog does not alert to any available nontargets	94% (with confidence interval of 87% to 98%) probability that the dog-handler teams will detect targets and correctly classify nontargets

Section 4. Discussion and Next Steps

The goal of this pilot study was to assess the efficacy and efficiency of using ecological scent-detection dogs to detect PPC, and the results suggest that detection dogs can potentially offer a method of survey that is more efficient than traditional transect surveys for the species. Two detection dogs were successfully trained to recognize the scent of PPC and to discriminate the scent from the scent of sympatric and related plant species. Through rigorous training and testing, the detection dogs demonstrated that they recognized the target scent when PPC was hidden in a container among control containers and when it was well camouflaged in a field setting. This target recognition remained strong even when the type of container and the location of the search area was manipulated and when potentially confusing nontarget odors were added to the training and trials. The detection dogs excelled at communicating the locations of targets to the handler, as demonstrated by 100% detection rates of the target during the scent recognition container and field tests, 80% and 95% detection rates of the target during the scent discrimination test, and 82% and 94% detection rates during field trials. This indicates that dog-handler teams have a high likelihood of detecting PPC at a site when the species is present, even in small numbers.

The detection dogs demonstrated exceptional scent discrimination abilities during scent discrimination training and testing, as well as during the field trials. The 98% success rate for discrimination achieved during the field trial instilled confidence in the handlers that the dogs would rarely alert to a nontarget in a true survey scenario. If a dog alerts to a nontarget on rare occasions, a handler trained to recognize the species would identify the false alert and withhold a reward, resulting in continued discrimination training and improved ability as more experience is gained. A discrimination score of 98% indicates that the dog will ignore scents that are not pertinent to the survey goals and maximize efficiency in a search scenario.

The results of this study are an important step in demonstrating that detection dogs may be able to provide a more efficient method of survey than traditional transect surveys. Additional testing in known PPC habitat is warranted to continue to explore how a detection dog will fare in actual survey conditions as compared to traditional visual surveys. In the Roller (1996) example, the surveyors found 100% as many PPC in the second and third surveys as in the first and did not find all the available PPC until the sixth survey. During the detection dog field trial, the dogs did not perform repeated surveys, but team 1 detected 100% of PPC available in three of five plots (one plot did not contain a target), and team 2 detected 100% of the PPC available in four of five plots. The two teams combined detected 30 out of 34 (88%) available targets with only one round of survey and, given this probability, have the potential to identify nearly 100% in two survey rounds; therefore, the detection rate with only one pass of the survey plots yielded a higher detection rate than at least three passes of a survey plot in one documented example of a traditional survey.

Although the targets were well camouflaged, the dogs were tested using healthy, mature plants that were all more than approximately 3 inches in diameter. Trial surveys in PPC habitat will illuminate how well the dogs detect seedlings and shriveled plants, as well as plants of all sizes. A large body of literature and data from our own detection dogs suggests that the dogs will find every variety of PPC at any stage of life and will particularly improve detection of PPC in the smallest size classes. Goodwin et al. (2010), for example, trained detection dogs to search for invasive spotted knapweed (*Centaurea stoebe*) in an area sparsely populated by the species. The detection dogs outperformed human surveyors overall (22% more accurate) but did so most dramatically with the medium-sized (16% more accurate) and small (200% more accurate) targets. In addition, at distances greater than 26 feet, detection dogs found 80% of small targets, whereas humans found 20%.

Similar results have been demonstrated with dogs detecting desert tortoises (*Gopherus agassizii*). Whereas dogs and humans achieved similar detection rates with large tortoises, dogs outperformed humans in detection of small tortoises. Dogs found tortoises with a carapace length as small as 30 millimeters, and the smallest tortoise found by a human had a 110-millimeter carapace length (Cablk and Heaton 2006). Furthermore, detection dogs detected tortoises at distances of up to 206 feet, and detection distance was independent of tortoise size (Cablk et al. 2008).

H. T. Harvey & Associates' ecological scent-detection dogs (Harvey Dogs) have demonstrated similar detection abilities, particularly for small avian fatality targets (Powers et al. 2015). During avian and bat fatality surveys, Harvey Dogs regularly detect partial specimens, such as a bird foot or single body feather or piece of membranous bat wing. This excellent detection ability results in more accurate avian fatality estimates and a greater chance of detecting a rare event with dogs than with human surveyors at renewable energy sites (Reyes et al. 2016). As demonstrated by these and other studies, detection dogs can offer more efficient surveys and greater accuracy, largely because of their ability to detect smaller targets at greater distances than human surveyors.

A potentially important characteristic of this target scent was observed during the training, testing, and trial phases of this study. The dogs appeared to direct their noses toward the root systems of the plants during training and trial. This suggests that most of the plants' odor, or the most reliable discriminatory odor, may emanate from the roots. Wild PPC searches in natural habitat will reveal whether this observation continues under natural conditions. If the dogs are indeed keying in to the root system as part of the odor profile, they will likely be proficient at detecting PPC at all stages of life. Goodwin (2005) tested dogs' ability to detect odor from whole spotted knapweed plants, plants that had been severed from the roots, and cut stems and leaves of the plant. The dogs demonstrated the ability to detect all parts of the plant during training. In addition, despite never being trained on dead or desiccated plants, the dogs alerted to such plants during field trials and appeared to direct their attention to the soil. It is suspected that the dogs learned to recognize the scent of the belowground chemicals produced by the roots as part of the odor profile of the plant. Long-term observations of the scenting behavior of the dogs revealed that the dogs appear to confirm the scent of the plant by sampling the soil surface before alerting to the target. Early observations of Harvey Dogs directing their scenting toward the roots of the PPC suggests that the roots may play a large role in the odor profile of this species, also.

Surveying for PPC using scent-detection dogs is an innovative application that may aid in the protection and recovery of the species. Further testing of this noninvasive, efficient, and effective survey technique is warranted because the method has great potential to support numerous efforts to recover and protect PPC. For example, detection dogs could survey for the species to identify suitable mitigation lands. Detection dogs also may provide a highly accurate and efficient survey technique for preconstruction clearance surveys, resulting in lower costs and high confidence that all PPC on the property will be detected and protected.

The results of this pilot study are encouraging and engender confidence that detection dogs offer a safe, efficient, and effective alternative survey technique to detect PPC. The recommended next step is to test the dog-handler teams' detection rate of naturally occurring PPC in known occupied habitat. Ideally, this test would be conducted in an area that was surveyed previously using current PPC survey methods so that presence of the target can be confirmed and the false positive rate can be quantified. Alternatively, the dog-handler team could perform surveys of an area before planned human survey efforts so that the results of the two methods can be compared. The H. T. Harvey & Associates' Harvey Dog program team looks forward to expanding on the results of this study and to future discussion and collaboration to further test this promising survey method.

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