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EFFORTS TO ERADICATE YELLOW CRAZY ANTS ON JOHNSTON ATOLL: RESULTS FROM CRAZY ANT STRIKE TEAMS X, XI AND XII (JUNE 2015–DECEMBER 2016)

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Abstract

Efforts to eradicate invasive yellow crazy ants (*Anoplolepis gracilipes*; YCA) on Johnston Atoll have been continuous since their discovery in 2010. Through 2014, a variety of commercial and novel formicidal baits were tested against the ant, but none proved capable of eradication. More recently, polyacrylamide crystals ("hydrogel") saturated with a sucrose solution containing the insecticide dinotefuran has been shown to be effective over large areas when applied against YCA alone or sequentially with a protein-based cat food bait. During June 2015–December 2016, Crazy Ant Strike Teams (CASTs) conducted treatment and monitoring efforts across an infestation of about 57 ha on Johnston Atoll. Following three infestation-wide treatments (primarily using hydrogel) during 2015, YCA were reduced 98% and surviving nests became difficult to find. Subsequently, a protocol designed to detect ants at low abundance that combined hand searching with a high density of baited monitoring stations (12 stations/0.25 ha; HST protocol) was employed within a network of 50 x 50 m cells that subdivided the infestation. During 2016 YCA were found at numerous locations using this method and standard grid-based bait monitoring surveys. Overall, 65 cells where YCA were detected, or cells adjacent to detections, were treated with hydrogel or cat food bait. YCA were not detected during four monitoring events each separated by at least one week, on 85% of these cells after 1–3 treatments, but it was necessary to treat several cells 4–7 times before YCA were eliminated. Results from HST searches allowed us to estimate the probability that YCA were detected when present in an area when searched using that method. Based on this probability, it was determined that areas would have to be searched three times without YCA being detected to allow 93% certainty that the ants were absent. The level of certainty increased to 99% when the search was conducted four times and YCA were not found. Overall, the likelihood of eradicating YCA on Johnston Atoll appears high using existing protocols.

Introduction

Yellow crazy ants (*Anoplolepis gracilipes*; YCA) are a highly invasive and ecologically destructive species. As generalist predators and competitors of a wide range of invertebrate and vertebrate taxa, they have been found to have negative impacts on numerous organisms in habitats in which they invade, including other arthropods (Holway et al. 2002, Lester and Tavite 2004, Abbott 2006), reptiles, and birds (Feare 1999, Matsui et al. 2009). Their ability to monopolize and manipulate populations of honeydew-producing insects or other carbohydrate sources has provided them with the energy to support colony sizes many times larger than ants with which they compete (Hill et al. 2003, Gerlach 2004). Ecological effects of these "supercolonies" can be profound and have been shown to alter ecosystem structure and function (O'Dowd et al. 2003). Believed to be native to moist tropical regions of Southeast Asia (Wetterer 2005) or Africa (Wilson and Taylor 1967), YCA are now widespread across many tropical and subtropical areas of the world.

YCA were first discovered by U.S. Fish and Wildlife Service (USFWS) staff on Johnston Atoll in January 2010. At that time, it was clear that YCA were impacting ground-nesting sea birds because nests were rarely found in areas were ants were abundant (USFWS unpublished data). Efforts to eradicate the ants from the island were immediate and the first Crazy Ant Strike Team (CAST) was deployed in August 2010. Initial mapping work determined that YCA occupied a contiguous area of about 38 ha. Since that time, CAST crews have been stationed on the island continuously in an effort to eradicate the ants. At maximum extent, YCA were estimated to cover 57 ha, or one-quarter of Johnston Island.

Because no formicidal bait was known to eradicate YCA at the level found on Johnston, early CAST crews focused on testing the efficacy of both commercially available and novel baits. No bait proved fully successful, but a novel bait developed during 2011 utilizing the active ingredient dinotefuran (Safari® SG Insecticide) mixed into a matrix of canned cat food, xanthum gum and corn syrup, was found to reduce the YCA population >90% (USFWS unpublished data). The reason that this bait failed to eradicate YCA is unclear, but may have been influenced by the ants identifying and rejecting the bait during subsequent applications, or their inability to carry the bait to the nest. The need for a more effective bait led to investigating alternative methods.

A new strategy initiated by CAST IX focused on developing a bait using inert polyacrylimide water-storing crystals ("hydrogel") saturated with a sucrose solution as the carrier of a formicidal insecticide (Peck et al. 2016). Small-plot tests of hydrogel bait containing dinotefuran against YCA on Johnston by CAST IX found it to be more successful than the cat food bait, although the results suggested that the two baits used sequentially over time also could be effective. Reasons for the success of hydrogel are unclear, but may be due to a lack of "bait shyness" that sometimes occurs with protein baits (Boser et al. 2014). Management alternatives proposed from results from CAST IX included developing an infestation-wide treatment strategy that focused on using the hydrogel-dinotefuran bait alone or in combination with cat food bait (Peck et al. 2016). It was further recommended that bait be applied across the infestation several times with intensive monitoring for surviving nests conducted between successive applications. Additionally, it was suggested that TVP (textured vegetable protein) may be considered as an alternative to hydrogel if the crystals accumulated on the ground to a level that warranted ecological concern.

This report summarizes the results of eradication efforts conducted by CAST X–XII during June 2015–December 2016. Below we briefly summarize the primary activities carried out during each CAST deployment. Adaptive management, driven by successes, failures, realizations and novel ideas, often guided the direction and accomplishments of each CAST. Additional details of work conducted by CAST X–XII are available in the form of weekly Situation Reports and final reports prepared at the end of each CAST deployment (USFWS unpublished reports).

CAST X tested whether the hydrogel bait developed during CAST IX would have the same success when applied over a larger area. The hydrogel bait was tested alone and in tandem with the standard cat food bait. Once found to be effective at a larger scale, these baits were applied across the entire infestation area. By the end of this deployment, YCA monitoring using non-lethal bait suggested that YCA abundance was very low and surviving nests were patchily distributed.

CAST XI developed techniques to locate YCA surviving at low densities and applied toxic bait in a manner that targeted individual nests. Extensive post-treatment monitoring revealed the success of targeted treatments. Infestation-wide surveys conducted during this time were used to calculate the detection probabilities of YCA using different survey methods and the survey effort required to accept that eradication was achieved with different levels of confidence.

CAST XII continued the intensive effort to locate and treat surviving YCA.

This report primarily provides a continuous narrative, but the activities of particular CAST crews are explained in some cases to help understand the timing or relevance of a particular action.

METHODS

Study Area

Johnston Atoll is located about 1,400 km southeast of Honolulu, Hawaii (16^{0}44′13″ N, 169^{0}31′26″ W). The atoll contains four islands, with Johnston Island being the largest, and only island on which YCA have been found. Originally about 19 ha in size, Johnston Island was expanded over several decades by the US Navy until it reached its current size of 241 ha in 1964. The three smaller islands range in size between 7 and 10 ha. Johnston Atoll was established as a federal bird refuge in 1926 and became part of the USFWS Pacific Remote Islands Marine National Monument in 2009. The refuge currently protects 11 species of nesting seabirds and provides terrestrial resting habitat for green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata*) turtles. The plant community consists of at least 46 native and introduced species (USFWS unpublished data) but is dominated by Indian fleabane (*Pluchea indica*), tree heliotrope (*Tournefortia agentea*), ironwood (*Casuarina equisetifolia*), haole koa (*Leucaena leucoephala*), kiawe or mesquite (*Prosopis pallida*) and beach naupaka (*Scaevola taccada*). Species such as sea grape (*Coccoloba uvifera*) and kou (*Cordia subcordata*) are relatively few in number but provide important habitat for YCA due to their relatively large size, deep root structure, flower-derived carbohydrate source, and ample litter production. The military deconstructed and removed most of the buildings from Johnston Island but a matrix of degrading roads, house pads and other unvegetated features remain. A centrally located 2.8 km long asphalt runway spans the long-axis of the island. The climate is subtropical and weakly seasonal with a mean annual temperature of about 27 ^{0}C and rainfall of 64 cm (data during 2010–2016; USFWS unpublished).

Consistent with earlier efforts, CASTs X–XII were each deployed on Johnston for approximately six months: CAST X was deployed June–December 2015, CAST XI was deployed December 2015–June 2016 and CAST XII was deployed June 2016–December 2016.

Identifying YCA distribution and abundance across the infestation

Initial patterns of abundance

YCA abundance and distribution were monitored during CASTs X–XII using methods developed and implemented by previous CAST expeditions. Soon after YCA were found on Johnston, the island was divided into 50 x 50 m cells to allow the infestation to be delimited and systematically surveyed. On this grid, permanent monitoring stations were placed at the southwest corners of each cell resulting in a network of about 1,050 stations, each 50 m from the nearest station; 314 of these stations fell within or immediately adjacent to the area occupied by YCA (Figure 1). Once efforts began to test and apply toxic baits to control YCA across the infestation, an additional array of stations was established to more effectively monitor the bait treatments (Figure 1). In contrast to the systematic 50-m array, these treatment monitoring stations were intentionally placed in areas where ants were most expected to be found; this resulted in a non-uniform distribution of about 490 stations placed in habitat considered to be favorable to ants, and largely avoided areas such as concrete and asphalt surfaces. During CAST XI, the number of treatment monitoring stations was increased to approximately 900 to further increase the likelihood of detecting ants. Each station was flagged, marked using a hand-held global positioning system (GPS) device, and provided with a permanent 10x10 cm ceramic tile on which non-toxic monitoring bait (Spam; Hormel Foods, Austin, MN) would be placed.

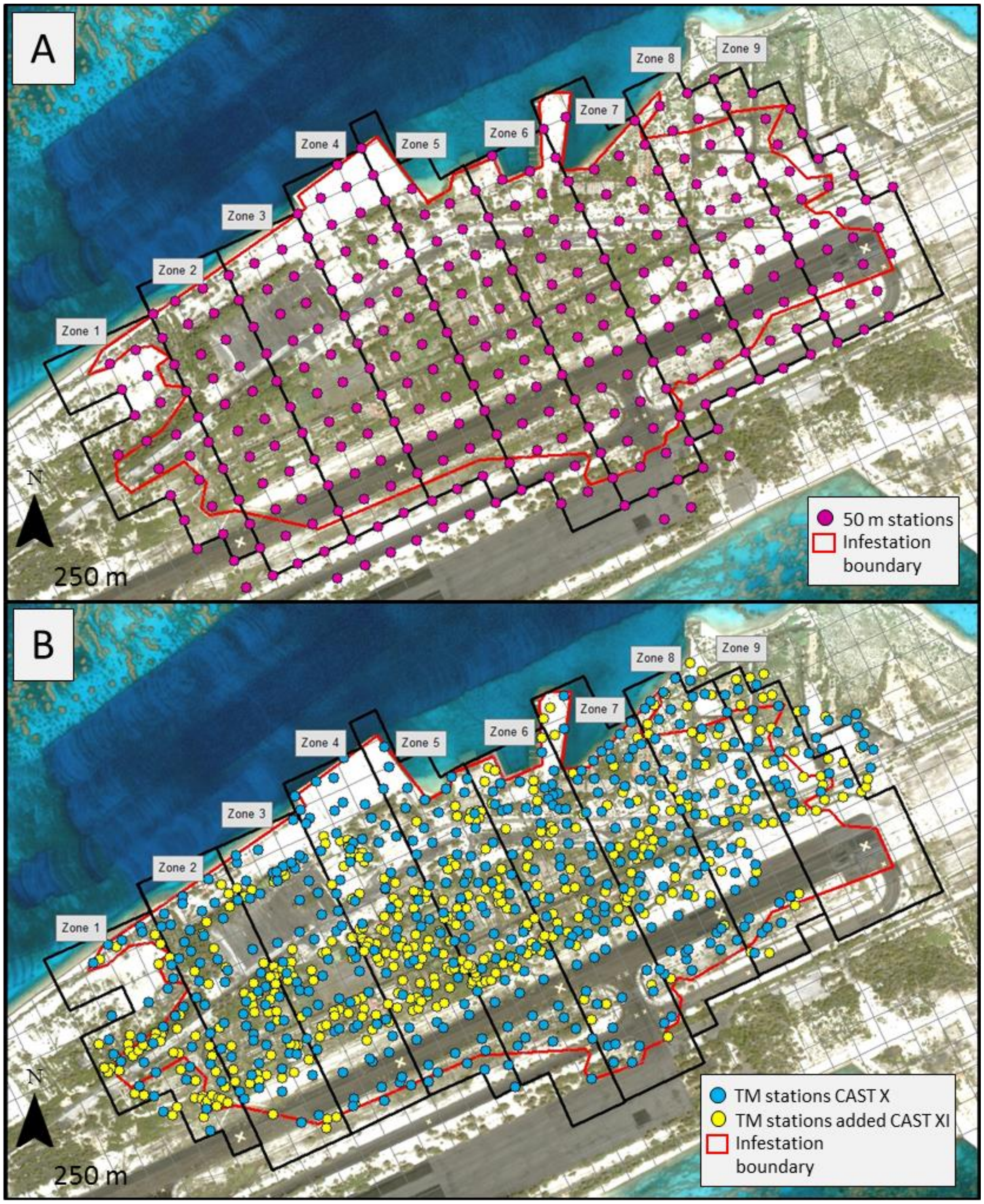

Figure 1. Locations of 50-m (A) and treatment monitoring (B) stations across the infestation area. Treatment monitoring stations established prior to, and used by, CAST X are indicated in blue and those stations added to the existing array by CAST XI are shown in yellow. The nine treatment zones are outlined in black.

Intervals between surveys sometimes varied, but 50-m surveys were conducted approximately every two weeks while treatment monitoring was conducted about every 2–4 weeks. During surveys, a pea-sized dollop of pureed Spam (1 part Spam to 1 part water) was placed on each tile and left to attract ants for about two hours. Surveys began around sunrise. At each station, YCA were counted and other ant species (OAS) were noted but not identified. The number of stations monitored during each survey often varied because bird nesting activity sometimes precluded access to certain areas.

Abundance patterns following bait treatment

To facilitate bait treatment during CAST X, the infestation was divided into nine zones, each comprised of 25–35 50 x 50 m cells (Figure 1). The zones were designed to include a buffer area of about 50-m beyond the maximum extent of the infestation that would provide confidence that nests that may have been missed during surveys were exposed to toxic bait during treatment. On several occasions, YCA were detected in cells bordering the outer edges of zones 1 and 9; therefore, several additional monitoring stations were established beyond the previously delimited treatment area.

A quadrat-based, hand-searching method was developed by CAST X to complement baiting with Spam during post-treatment monitoring. The quadrat assessment consisted of placing a 0.25 m^2 frame within about 1 m of treatment monitoring stations and counting the number of YCA seen moving within the frame during 1–minute intervals. No Spam bait was used to attract ants, and counts were postponed at a station for several days following a Spam baited count. Quadrat counts were conducted within about 2.5 hours after sunrise and took place approximately 7–10 days following each bait application. Time constraints toward the end of the CAST X deployment, combined with the efficiency of treatment monitoring, resulted in quadrat assessment of baits following the third application being limited to zones 1–6.

Although 50-m, treatment monitoring, and quadrat surveys are effective at assessing YCA populations at high densities, they are less effective at detecting YCA when ant densities are low. As a result, a more intensive method was developed for finding YCA once their abundance had been greatly reduced by hydrogel and cat food bait. This method involved a simultaneous effort that employed both hand searching and a large number of baited tiles. This method became known as the HST (hand searching combined with bait tiles) protocol. Beginning with CAST XI, HST surveys were used to systematically search several cells each day; over time, HST searching swept sequentially across the infestation area, one zone at a time.

Each day that HST surveys were conducted, four CAST members hand searched for YCA while the fifth member serviced the bait stations. The four hand searchers simultaneously walked along and surveyed an approximately 12.5 m wide swath across each cell. Searching included visually examining the ground and vegetation (including the trunk, branches, foliage, flowers and fruit), turning over coral rubble and coarse woody debris, and lightly disturbing the litter. Each cell was searched for a minimum of 15 minutes (60 person-minutes); areas with particularly dense vegetation were searched for longer periods. After a minimum of 15 minutes, the team moved to the next cell and began a new search.

During HST surveys, 12 bait stations were placed within each cell. Baiting at stations was conducted in a fashion similar to hand searching except that all tiles throughout the set of cells to be searched that day were baited consecutively prior to assessment. Because the relative attractiveness of Spam puree and hydrogel (mixed with water and sugar) was not known, and their attractiveness was suspected to vary over time and among nests, both baits were placed adjacent to each other on tiles. Once all tiles were baited, they were allowed to attract ants for approximately two hours. After that time, the number of YCA present was recorded and the occurrence of other ant species noted. HST surveys were generally conducted during the first four hours after sunrise, before ant activity was reduced by high temperature.

Locations where YCA were detected during HST surveys were flagged and marked using GPS units. Later, the area around each detection point was searched intensively in an attempt to find nest locations. This information was subsequently used for treating and monitoring the area.

Because YCA nests were often located at the base of trees and shrubs or along concrete slabs surrounded by dense vegetation, preparation for HST surveys often took considerable time and effort. In many cases, this involved clearing vegetation to allow entry to difficult-to-access locations, and to create paths on which to efficiently traverse the cells. Following this effort, the 12 tiles were placed within each cell at locations where YCA were most likely to be found, such as adjacent to bases of plants and under their canopies.

The first complete infestation-wide HST survey was conducted by CAST XI over 28 work-days between 21 January and 20 April 2016. The methodology and results were evaluated following this survey, and it was determined that hand searching was considerably more effective than baiting with Spam because few detections occurred by baiting alone. Therefore, only hand searching was performed during the second survey conducted after May 2016.

In addition to surveys within the infestation area, island-wide ant surveys based on the 50-m grid were conducted once during each CAST deployment to determine whether YCA had expanded beyond the recognized infestation area. During this survey, a 15 ml plastic vial containing a small cube of intact Spam (approximately 1 cm^3) was placed on the ground around sunrise and allowed to attract ants for about two hours. Upon collection, each vial was capped and frozen until ants could be identified. A total of about 1,050 Spam-baited stations comprised each survey. In addition to the island-wide survey, an attempt was made to survey the three small islands within the lagoon of the atoll during the five days when CAST crews were exchanged. However, the outer islands were accessed using a tender boat from the MV Kahana and were only accessible when sea conditions were favorable. Ants were surveyed on those islands using the Spam-in-vial method used during the island-wide survey.

Formicidal bait formulation and application

The recipes for formicidal baits applied during CAST X–XII followed those developed or used by CAST IX (Peck et al. 2016). The following ingredients were used to make about 1 kg of hydrogel bait containing dinotefuran at 0.05%: 786 ml water, 196 g sucrose, 15.6 g Soil Moist crystals (JMR Chemical, Cleveland, OH), and 2.0 g Safari 20 SG Insecticide (Valent Corporation, Walnut Creek, CA). The amount of water and crystals listed here differed slightly from that used by CAST IX because Soil Moist crystals replaced the Miracle-Gro crystals (Miracle-Gro Lawn Products, Marysville, OH) used previously. The ingredients used to make 1 kg of cat food bait included 250 g canned cat food (Friskies®, Nestlé Purina, St. Louis, MO), 12.5 ml Xanthan gum, 250 g Karo dark corn syrup (ACH Food Companies, Inc., Cordova, TN), 500 ml water, and 2.5 g Safari 20 SG Insecticide. This mixture produced a dinotefuran concentration of 0.05%. The ingredients used to make 1 kg TVP bait containing dinotefuran at 0.05% included: 615 ml water, 154 g sucrose, 231 g TVP (Honeyville®, Brigham City, UT), and 1.54 g Safari 20 SG Insecticide.

The standard rate at which hydrogel, cat food and TVP bait were applied was 60.8 kg/ha. However, this rate was reduced to 20 kg/ha within individual cells or treatment areas when <25% of the ground surface area was covered by vegetation; areas of reduced bait volume generally included a significant amount of asphalt or concrete (e.g. roads, runway or remnant building pads).

CAST X completed three infestation-wide applications of bait. The total area over which toxic bait could be applied each day varied with bait type, habitat structure, and CAST experience, but generally ranged from 12–18 cells per day. Bait was generally prepared in then early to midafternoon and applied in the late afternoon or early evening. A critical protocol was to apply bait when temperatures were relatively cool and rainfall amounts low. Table 1 shows the dates during which hydrogel, cat food, and TVP were applied in each zone.

The method by which bait was applied varied among types. Hydrogel bait was primarily distributed using the "dip and flick" method whereby a handful of sticky hydrogel was scooped from a bucket using a gloved hand and tossed at desired locations across the cell. Hydrogel globs generally broke into smaller clusters upon impact with vegetation or the ground. The less viscous cat food bait was distributed using a Stream Machine QF-2000 Water Launcher (Stream Machine, Palatine, IL) to slowly dispense bait while walking across the treatment areas. TVP bait was applied by spreading the saturated granules around the cells by hand. The amount of bait required to treat each cell was placed in a bucket prior to application and that amount was distributed as uniformly as possible across the cell.

Table 1. Bait type and date of application within nine treatment zones during three rounds of treatment during August–November 2015.

	Round 1		Round 2		Round 3	
Zone	Bait type	Application date	Bait type	Application date	Bait type	Application date
1	hydrogel	29—31 Jul, 3 Aug	hydrogel	10–12 Aug	hydrogel	19–23 Oct
2	hydrogel	29–31 Jul, 3 Aug	hydrogel	10–12 Aug	hydrogel	19–23 Oct
3	cat food	3–6 Aug	hydrogel	17–20 Aug	hydrogel	19–23 Oct
4	cat food	3–6 Aug	hydrogel	17–20 Aug	hydrogel	28–31 Oct
5	cat food	31 Aug–3 Sep	hydrogel	14-15 Sep	TVP	28-31 Oct
6	cat food	31 Aug–3 Sep	hydrogel	2, 5–9 Oct	hydrogel	5–6 Nov
7	cat food	7–8 Sep	hydrogel	5–9 Oct	hydrogel	16–19 Nov
8	hydrogel	5–9 Oct	hydrogel	19–23 Oct	hydrogel	16–19 Nov
9	hydrogel	5–9 Oct	hydrogel	19–23 Oct	hydrogel	16–19 Nov

Following completion of an infestation-wide HST survey by CAST XI, all zones were treated with hydrogel during 25 April–12 May 2016. Exceptions to this treatment were 10 cells where cat food was scheduled to be applied because hydrogel had been applied on two occasions earlier as part of a targeted eradication effort.

Targeted bait treatment and monitoring
Following the CAST X zone-wide applications of hydrogel and cat food, treatment monitoring surveys revealed that YCA survived patchily across the infestation. As a result, it became more efficient to treat only areas where ants had been detected, rather than entire zones. Baiting that targeted individual YCA detections was initiated toward the end of CAST X and employed fully during CAST XI. An exception to this strategy was the infestation-wide application made by CAST XI made as a precaution to expose YCA that may have been missed during surveys to toxic bait. The amount of area treated at that time depended upon the location of the YCA detection. For example, if the detection was located in the center of the cell, then only that cell was treated. However, if the detection was located near a cell border, then the adjacent cell was also treated. And if the detection was made at a cell corner (as happened during 50-m surveys), then all four cells surrounding the station were treated. Successful efforts to locate nests further enhanced the efficiency of treatment because both baiting and monitoring could be more focused. During CAST XII, the targeted treatment strategy was sometimes modified to be yet more efficient, with bait applied only within a 20-m radius around a nest or detection point rather than within one or more complete cells. The amount of bait applied in this fashion was calculated to maintain the application rate of 60.8 kg/ha and approximately one-half that applied across a 50-m cell.

The targeted eradication strategy was based on a treat-monitor-treat-monitor approach until YCA were no longer found around the detection point. Once YCA were detected, hydrogel bait was applied to the infested area. Post-treatment monitoring was then conducted within 7–10 days. All substrates within the treated area were searched for surviving YCA but the search focused around the original detection point, or nest location, if known. If YCA were present at that time, then the area was treated a second time with hydrogel and the monitoring protocol was repeated. If a third bait application was required to eradicate YCA, then cat food often replaced hydrogel as the bait. In cases where ants survived the third treatment, bait was suspended for a minimum of three weeks (a "rest" period) before reinitiating the treatment process. YCA were considered eradicated from the treatment area if they were not detected during four consecutive post-treatment monitoring events spaced at least one week apart. In a few instances, YCA were detected during treatment monitoring or HST surveys conducted after four treatment monitoring searches suggested that they had been eradicated. In these cases, treatment protocols were renewed in those cells. In areas where YCA were particularly difficult to eradicate, flowers were removed from plants to reduce competition for sugar resources.

YCA detection probability and occupancy analysis
Although it is nearly impossible to determine whether eradication has been achieved in an area over the short-term, a degree of confidence surrounding the potential for eradication can be estimated. This probability can be a valuable tool for developing a treatment and monitoring strategy. A critical question to be determined is, "How many times does an area need to be surveyed before it can be declared free of ants?" Answering that question depends upon how readily YCA are detected and how certain managers wish to be when they make that declaration.

The minimum number of times an area (e.g. 50 x 50 m cell) needs to be searched to provide a high degree of certainty that ants are no longer present depends upon 1) the probability of detecting ants if they are present, and 2) the acceptable rate of error (the chance of erroneously declaring an area free of ants). For example, if the probability of detecting ants within an area is 0.5 (that is, ants are detected 50% of the time each area is searched when ants are present) and the desired level of certainty that ants are not present is 90% (acceptable error rate of 10%), then the minimum number of repeated, independent searches of the area is five (MacKenzie et al. 2006). If no ants are detected during the five searches, then there is at least a 90% probability that no ants are present in the area. As the probability of detecting ants increases, the number of searches required to meet a predetermined level of precision decreases.

To estimate the probability of detecting YCA and to estimate the effort required to declare eradication with certainty, data from HST surveys (methods described above) conducted on zones 1–6 during 22 January–10 March 2016 were analyzed. The HST surveys used both hand collection and bait stations, which allowed the relative effectiveness of the two methods to be compared. The detection probabilities were estimated using a double occupancy model with the "unmarked" software package (Fiske and Chandler 2011) in the R statistical environment (R Core Team 2016). With the detection probabilities for each method, we projected how sampling effort influenced our ability to detect YCA across the infestation area (250 cells north of the runway). This was done by multiplying the initial detection probabilities by the number of cells to be surveyed by the number of repeated surveys conducted. We assumed that the detection probabilities were constant across the infestation area.

Contact insecticide trials

Contact insecticides are effective at killing ants and other insects when they are directly exposed to the toxins. Ants can be exposed to the insecticide by drenching soil harboring their nests with insecticide suspended in water or by dusting nest entrances and foraging trails with insecticidal powder. To enhance the ability to kill YCA on Johnston, particularly queens and their brood within nests, the efficacy of several contact insecticides was tested on captive YCA nests maintained in the lab. The goal of experimental trials conducted by CAST XII was to determine survival rates of queens and workers exposed to both liquid and dry insecticides. The following four drench insecticides were tested: Demon® Max Insecticide (cypermethrin 25.3%; Syngenta Crop Protection, Greensboro, NC); Bifen I/T (bifenthrin 7.9%; Control Solutions Inc., Pasadena, TX); Suspend® SC (deltamethrin 4.75%; ArgEvo Environmental Health, Montvale, NJ); and Permethrin SFR (permethrin 36.8%; Control Solutions Inc., Pasadena TX). In addition, the dry powder Terro Ant Dust (deltamethrin 0.05%; Senoret Chemical Company, Lititz, PA) was tested.

Insecticides were tested in ways simulating conditions of exposure on Johnston. The first trial tested the impact of direct exposure that would take place either through soil drenching or the dusting of a nest entrance. For the liquid baits, 0.8 ml of diluted insecticide was pipetted onto a cotton ball placed into a 15 ml conical centrifugal tube in which a single YCA worker had been placed. The application of Delta Dust was similar except roughly 2.5 ml of powder was gently blown into the tube containing a worker ant using a handheld insecticide bulb duster. Ants contacted the insecticides by walking around inside the vials. Once ants were observed making contact with the insecticides, the amount of time required for ants to die was recorded. The experiment was conducted once for Bifen I/T, Permethrin SFR and Suspend SC and twice for Demon Max and Delta Dust. Each insecticide was applied at concentrations recommended on the label. The experiment took place during 7–15 July 2016.

The second trial was designed to replicate exposure that may exist when ants walk over soil or other substrates containing the pesticide. Exposure could be from a liquid suspension that saturated underground passageways or could be from dry insecticide that was dusted onto trails. In this test, 8 ml of each liquid insecticide and about 7.5 ml of Delta Dust were thoroughly mixed into 120 ml of soil, and portions of the soil placed loosely into one end of the tubes (about 1 cm diameter) that spanned between the nest boxes and the feeding arenas (see Peck et al. 2016 for a description of the nest boxes). Worker ants were forced to push through the contaminated soil to gain access to food. Queen ants rarely left their nest box so their exposure to the insecticide was expected to be from workers harboring insecticide that return to the nest; exposure could be from direct ant-ant contact or to insecticide groomed from the bodies of worker ants that remained in the nest. The number of surviving queen and worker ants were counted one or two times per day during 7–15 July 2016. Each insecticide was tested against three nests.

RESULTS

YCA distribution and abundance

Initial patterns of abundance

YCA were widespread and abundant throughout much of the infestation area immediately prior zone-wide application of toxic bait by CAST X (assessed during mid–late July 2015), particularly north of the runway (Figure 2). At that time, YCA were detected at 93 (29.6%) of the 314 50-m stations and 228 (47.3%) of the 482 treatment monitoring stations. However, if stations south of the runway, where YCA were rarely found, are excluded, then the percent of stations where YCA were detected was 41.9% (93 of 222) at 50-m stations and 56.9% (228 of 401) at treatment monitoring stations. Both survey types indicated that YCA were primarily found in zones 2–8 as ants were only detected at three stations in zone 1 and seven stations in zone 9. Considering only stations where YCA were detected, the mean number of YCA counted at 50-m stations was lower than at treatment monitoring stations (23.2±1.2 SEM and 31.8±0.7 SEM, respectively; t-test: $t=6.1$, $P<0.001$; Table 2). At those stations, mean YCA abundances within zones ranged from 14.7 to 35.0 at 50–m stations and 20.5 to 35.5 at treatment monitoring stations. Over all treatment monitoring and 50-m stations within the infestation area, other ant species (OAS) were found at 321 stations, and 18 stations contained both YCA and OAS.

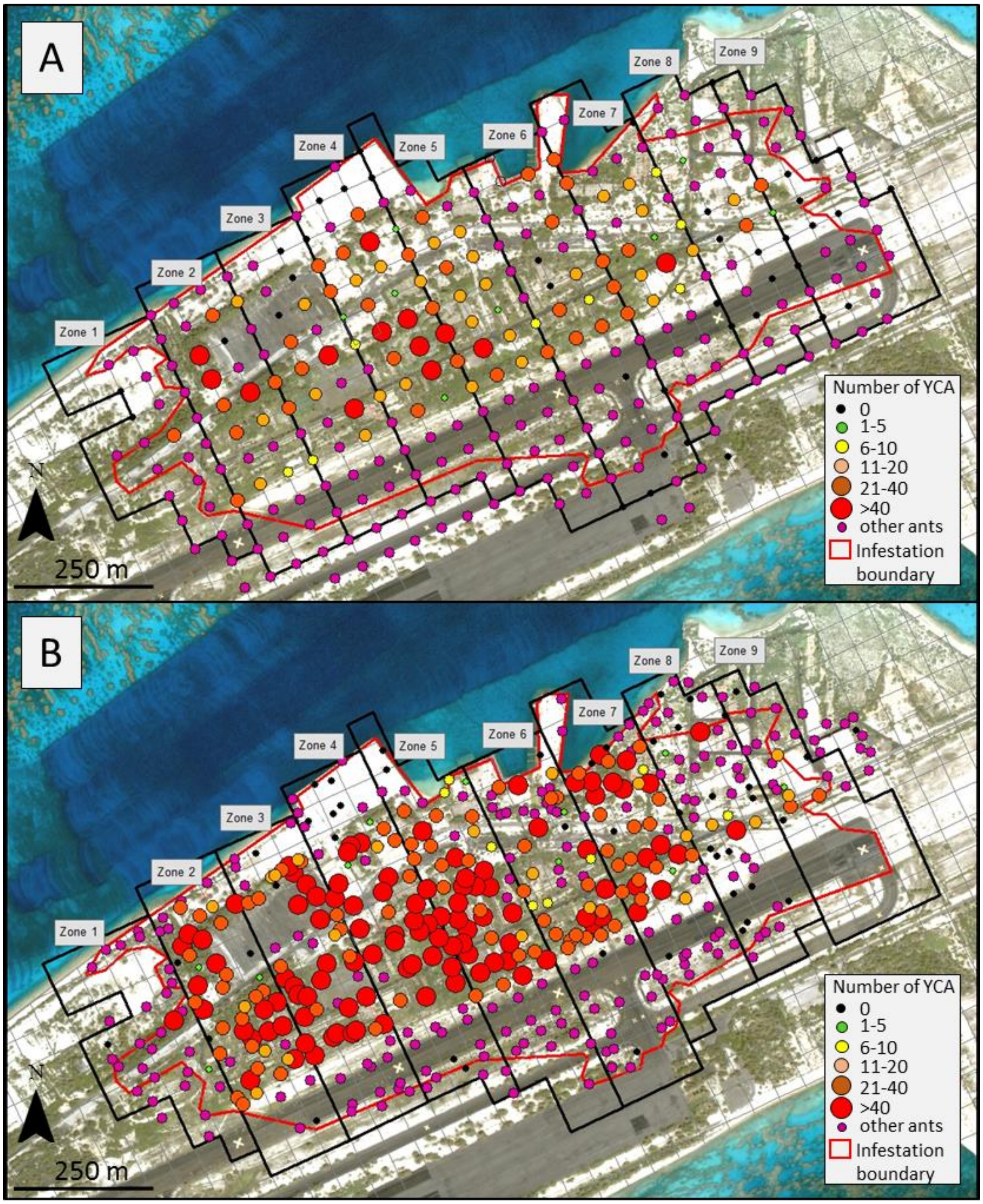

Figure 2. Distribution and abundance of YCA detected during 50-m (A) and treatment monitoring (B) surveys prior to zone-wide bait treatment by CAST X. 50-m and treatment monitoring surveys were conducted on 20 and 27 July 2015, respectively.

Table 2. Mean (±SEM) number of YCA counted at 50-m and treatment monitoring stations where YCA were present within the nine zones located in the infestation area during 20–27 July 2015.

	50-m			Treatment monitoring		
Zone	Number of stations	Mean	SEM	Number of stations	Mean	SEM
1	1	35.0		2	20.5	
2	10	29.5	3.7	39	31.7	1.6
3	11	26.2	3.3	37	34.7	1.5
4	20	24.1	3.1	31	35.3	1.9
5	20	20.9	2.5	44	33.2	1.6
6	9	23.4	3.3	30	26.4	2.3
7	14	22.1	2.8	34	32.4	1.7
8	6	14.7	4.3	6	21..3	6.2
9	2	17.0	13.0	5	21.0	4.6
Total	93	23.2	1.2	228	31.8	0.7

Abundance patterns following zone-wide toxic bait treatment

Post-treatment YCA surveys using quadrats showed that both hydrogel and cat food bait reduced the number of detection points and ant abundances when applied across zones, although hydrogel had a greater impact. During the first round of treatment, the number of stations at which YCA were detected was reduced 63.7% (range 37.9–75.0%) in the five zones where cat food was applied and 94.0% (range 83.8–100%) in the four zones treated with hydrogel (Table 3). Similarly, the total number of YCA counted in quadrats following treatment was 77.7% (range 65.0–97.8%) lower in zones where cat food was applied and 99.0% (97.5–100) lower in zones receiving hydrogel (Table 4).

Overall, the application of hydrogel across all zones during the second application of bait further reduced YCA presence and abundance. YCA detection rate in quadrats across all zones dropped 93.9% relative to initial detection rates, and no YCA were detected in four zones (Table 3). Across all zones, YCA were detected at only 13 stations. Counts of YCA declined by 84.1% across all zones following the first application of baits and by 96.5% following the second application (Table 4).

YCA presence and abundance continued to decline following the third round of zone-wide bait application. For the six zones in which quadrats counts were conducted, the number of stations at which YCA were detected decreased by 96.7% compared to initial rates of station occupancy (Table 3). Similarly, the mean reduction in numbers of YCA counted in quadrats decreased by 97.9% (Table 4). Although YCA were not eliminated from five stations where TVP was applied during the third round of treatment, the total number of ants fell from 59 to 25 (56.6% reduction).

Post-monitoring assessments using quadrats were largely corroborated by 50-m and treatment monitoring surveys conducted during the beginning of CAST XI (December 2015; Figure 3). YCA were detected at one location each in zones 5 and 6 during 50-m surveys. During the treatment monitoring survey one week later, YCA were detected at seven stations, with one detection in zone 2 and two detections each in zones 4, 5, and 7. One of the detections made during treatment monitoring was within about 25 m of the detection made in zone 6 during the 50–m survey, suggesting that YCA from the same nest were detected on both occasions. For 50–m and treatment monitoring combined, the number of stations at which YCA were detected fell from 288 to 9 (96.9% decrease) and the number of YCA counted fell from 7,322 to 196 (97.3% reduction).

YCA assessments made during 50-m and treatment monitoring surveys conducted between December 2015 and December 2016 consistently found the frequency at which ants were detected to be low (Figure 4). During 50-m surveys, YCA were found only on and 18 Jan, 2016 (at three and two stations, respectively), and were not detected during the ten subsequent surveys conducted between 16 Feb and 2 Nov, 2016. Treatment monitoring detected YCA more frequently and over a longer period than 50–m surveys as ants were found on 8 of 13 treatment monitoring surveys performed during that time. Overall, the mean number of stations at which YCA were detected during these treatment monitoring events was 3.4 (±1.3).

Table 3. Number of stations at which YCA were detected in quadrats within the nine treatment zones following three rounds of treatment. The percent reduction in the number of stations where YCA were detected during each round is relative to a pre-treatment survey conducted on 28 July 2015.

		Round 1			Round 2			Round 3		
Zone	Pre-treatment	Bait type	Post-treatment	% YCA reduction	Bait type	Post-treatment	% YCA reduction	Bait type	Post-treatment	% YCA reduction
1	1	hydrogel	0	100.0	hydrogel	0	100.0	hydrogel	0	100.0
2	37	hydrogel	6	83.8	hydrogel	1	97.3	hydrogel	0	100.0
3	36	cat food	10	72.2	hydrogel	0	100.0	hydrogel	1	97.2
4	29	cat food	18	37.9	hydrogel	5	82.8	hydrogel	1	96.6
5	41	cat food	17	58.5	hydrogel	5	87.8	TVP	5	87.8
6	24	cat food	6	75.0	hydrogel	0	100.0	hydrogel	0	100.0
7	28	cat food	7	75.0	hydrogel	2	92.9	hydrogel	–[1]	–[1]
8	13	hydrogel	1	92.3	hydrogel	0	100.0	hydrogel	–[1]	–[1]
9	5	hydrogel	0	100.0	hydrogel	0	100.0	hydrogel	–[1]	–[1]
All zones	214		65	69.6		13	93.9		7	96.7

[1]No post-treatment quadrat survey was conducted

Table 4. Number of YCA counted in quadrats within the nine treatment zones following three rounds of treatment. The percent reduction in YCA abundance is relative to a pre-treatment survey conducted on 28 July 2015.

		Round 1			Round 2			Round 3		
Zone	Pre-treatment	Bait type	Post-treatment	% YCA reduction	Bait type	Post-treatment	% YCA reduction	Bait type	Post-treatment	% YCA reduction
1	4	hydrogel	0	100.0	hydrogel	0	100.0	hydrogel	0	100.0
2	353	hydrogel	9	97.5	hydrogel	3	99.2	hydrogel	0	100.0
3	510	cat food	11	97.8	hydrogel	0	100.0	hydrogel	1	99.8
4	541	cat food	128	76.3	hydrogel	18	96.7	hydrogel	25	95.4
5	598	cat food	117	80.4	hydrogel	59	90.1	TVP	25	95.8
6	167	cat food	52	68.9	hydrogel	0	100.0	hydrogel	0	100.0
7	206	cat food	72	65.0	hydrogel	6	97.1	hydrogel	–[1]	–[1]
8	61	hydrogel	1	98.4	hydrogel	0	100.0	hydrogel	–[1]	–[1]
9	12	hydrogel	0	100.0	hydrogel	0	100.0	hydrogel	–[1]	–[1]
All zones	2452		390	84.1		86	96.5		51	97.9

[1]No post-treatment quadrat survey was conducted

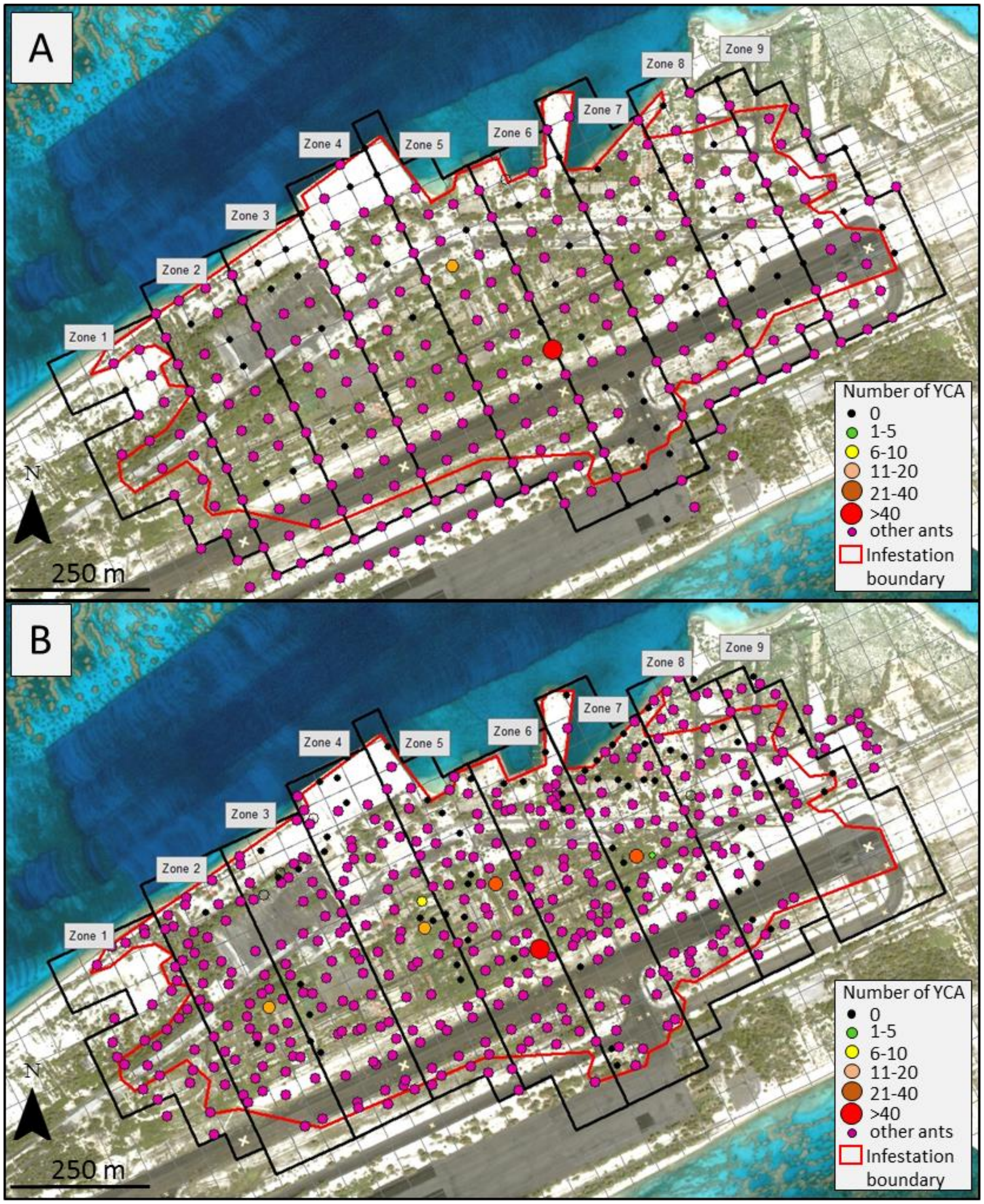

Figure 3. Abundance of YCA detected during 50–m (A) and treatment monitoring (B) surveys within the nine treatment zones at the beginning of CAST XI. Treatment monitoring and 50-m surveys were conducted on 28 and 21 December 2015, respectively.

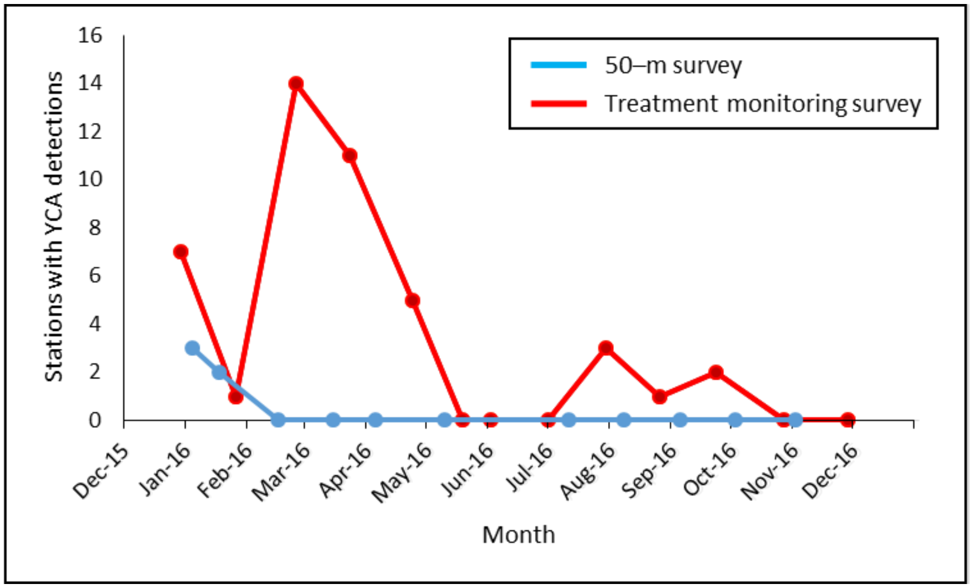

Figure 4. Number of stations at which YCA were detected during 50-m and treatment monitoring surveys conducted between 28 December 2015 and 28 November 2016 (CASTs XI and XII). The number of bait stations monitored during 50-m and treatment monitoring surveys were about 310 and 900, respectively.

During HST surveys, YCA were detected in 29 cells within eight zones during the first round conducted during 21 Jan–17 Apr 2016. The number of detections ranged from zaro in zone 9 to seven in zone 4. In contrast, a second complete round of HST surveys conducted during 22 May–13 Nov 2016 resulted in YCA being detected in only one cell in zone 8. No YCA were found within zones 1–7 or 9.

Targeted treatment and monitoring

Between December 2015 and December 2016, 65 individual cells were treated on one or more occasions with hydrogel or cat food bait (Figure 5). YCA had been detected in many of these cells, but on some instances adjacent cells were also treated since the actual location of the nests supporting these ants were sometimes not found. The number of times bait needed to be applied to cells before YCA were no longer detected varied widely, ranging from a single application (29 instances) to seven applications (one instance; mean = 2.2; Table 5). Across all 65 cells, hydrogel was applied 128 times and cat food was applied 13 times. In cells requiring >2 applications to eradicate ants, cat food was applied most frequently during the third application (11 out of 22 treatments). Based on the third application, hydrogel was slightly more effective at eradicating ants than cat food, as it was the final bait applied on seven occasions compared to five times for cat food.

Figure 5. Cells on which were YCA were treated by CASTs XI and XII during December 2015–December 2016. Note that several cells were treated by both CASTs.

Table 5. Number of applications of toxic bait until YCA were no longer detected after four surveys during targeted treatment. The left half of the table shows the number of cells to which each bait type (hydrogel or cat food) was applied during each application (1–7 applications). The right half of the table shows the frequency at which each bait represented the final treatment (i.e., no YCA were found in a cell following that application) during each application. Data are based on YCA detected, treated and monitored during Dec 2015–Dec 2016.

		Bait type			Final bait type	
Application number	Number of cells treated	Hydrogel	Cat food	Number of applications before YCA not found	Hydrogel	Cat food
1	65	65	0	29	29	0
2	36	35	1	14	14	0
3	22	11	11	12	7	5
4	10	9	1	4	4	0
5	6	6	0	5	5	0
6	1	1	0	0	0	0
7	1	1	0	1	1	0
Total	141	128	13	65	60	5

YCA detection probability and occupancy analysis
YCA were detected on 28 of 160 cells (17.5%) in zones 1–6 during HST surveys conducted during January–March 2016 (Table 6). Of these, ants were found on 27 cells by hand searching and 16 cells using baited tiles; on only one cell was YCA detected using bait tiles and not found by hand searching. On cells where YCA were detected using bait, the number of tiles at which ants were found ranged from 1–5 (of 12 total).

Based on HST results, occupancy analysis quantified the difference between hand searching and baiting in terms of how effectively YCA were detected using each method. The probability of detecting YCA when they were known to be present by hand searching during a single search of a cell was 0.93 (95% CI = 0.99–0.65) compared to 0.54 (95% CI = 0.71–0.35) using bait tiles. Overall, these results suggest that hand searching is 72% more effective at finding YCA than bait tiles.

In order to make an inference about the entire infestation north of the runway (250 cells) we need to consider the cumulative chance of making a correct detection in each of 250 cells. For both methods there was effectively 0% chance of correctly detecting ants in all 250 cells after a single survey (Figure 6). However, the probability of missing YCA in all cells decreased to 67% after a second hand search and to 7% after a third search. In contrast, ten repeated surveys were required to attain a confidence level of 90% that no YCA were missed during baiting with Spam.

Contact insecticide trials
Direct exposure of YCA workers to the five contact insecticides placed in centrifuge tubes resulted in ant death relatively rapidly in all cases. Overall, the mean amount of time elapsed before ants died was 10.8 min (±1.4) and ranged from 6.5 minutes for Demon Max (2.6 and 10.4 min for the two trials) to 14 minutes for Bifen I/T.

Soil in which contact insecticide had been mixed and placed in tubes connecting nests and feeding arenas affected YCA queens and workers in different ways (Figure 7). None of the insecticides reduced the number of queens over the duration of the study. In contrast, four of the five insecticides reduced mean worker abundance to a high degree over time. Delta Dust had the strongest impact, reducing YCA abundance 75.7%. The effect of Bifen I/T and Permethrin SFR was roughly similar, reducing YCA worker abundance by 46.2 and 40.7%, respectively, while Demon Max reduced worker numbers by 28.9%. Only Suspend SC failed to impact YCA worker ants (3.4% reduction).

Table 6. Survey data used to estimate YCA detection probability. The table shows the cells (50 x 50 m) in which YCA were detected by hand searching and using tiles baited with Spam during HST surveys conducted January–March 2016. Overall, 160 cells were surveyed across six zones.

			Detection method		
Date	Zone	Cell	Hand searching	Bait tiles	Number of bait tiles with YCA
22-Jan-16	1	31H	Yes	Yes	1
27-Jan-16	1	33J	Yes	Yes	1
27-Jan-16	1	33K	Yes	No	0
8-Feb-16	2	36J	Yes	No	0
8-Feb-16	2	34J	Yes	Yes	2
9-Feb-16	3	38D	Yes	No	0
9-Feb-16	3	38E	No	Yes	1
10-Feb-16	3	39H	Yes	No	0
10-Feb-16	3	37H	Yes	No	0
12-Feb-16	3	37J	Yes	Yes	1
12-Feb-16	3	38J	Yes	No	0
12-Feb-16	4	40J	Yes	No	0
12-Feb-16	4	41J	Yes	Yes	2
12-Feb-16	4	42J	Yes	No	0
19-Feb-16	4	41E	Yes	No	0
25-Feb-16	4	41G	Yes	Yes	5
25-Feb-16	4	40G	Yes	Yes	1
25-Feb-16	4	41H	Yes	Yes	2
2-Mar-16	4	42I	Yes	No	0
2-Mar-16	5	45J	Yes	No	0
2-Mar-16	5	44K	Yes	Yes	1
3-Mar-16	5	45E	Yes	Yes	1
3-Mar-16	5	45F	Yes	Yes	1
4-Mar-16	5	43H	Yes	Yes	2
4-Mar-16	5	44H	Yes	Yes	1
7-Mar-16	6	47G	Yes	No	0
10-Mar-16	6	48J	Yes	Yes	1
10-Mar-16	6	48K	Yes	Yes	2

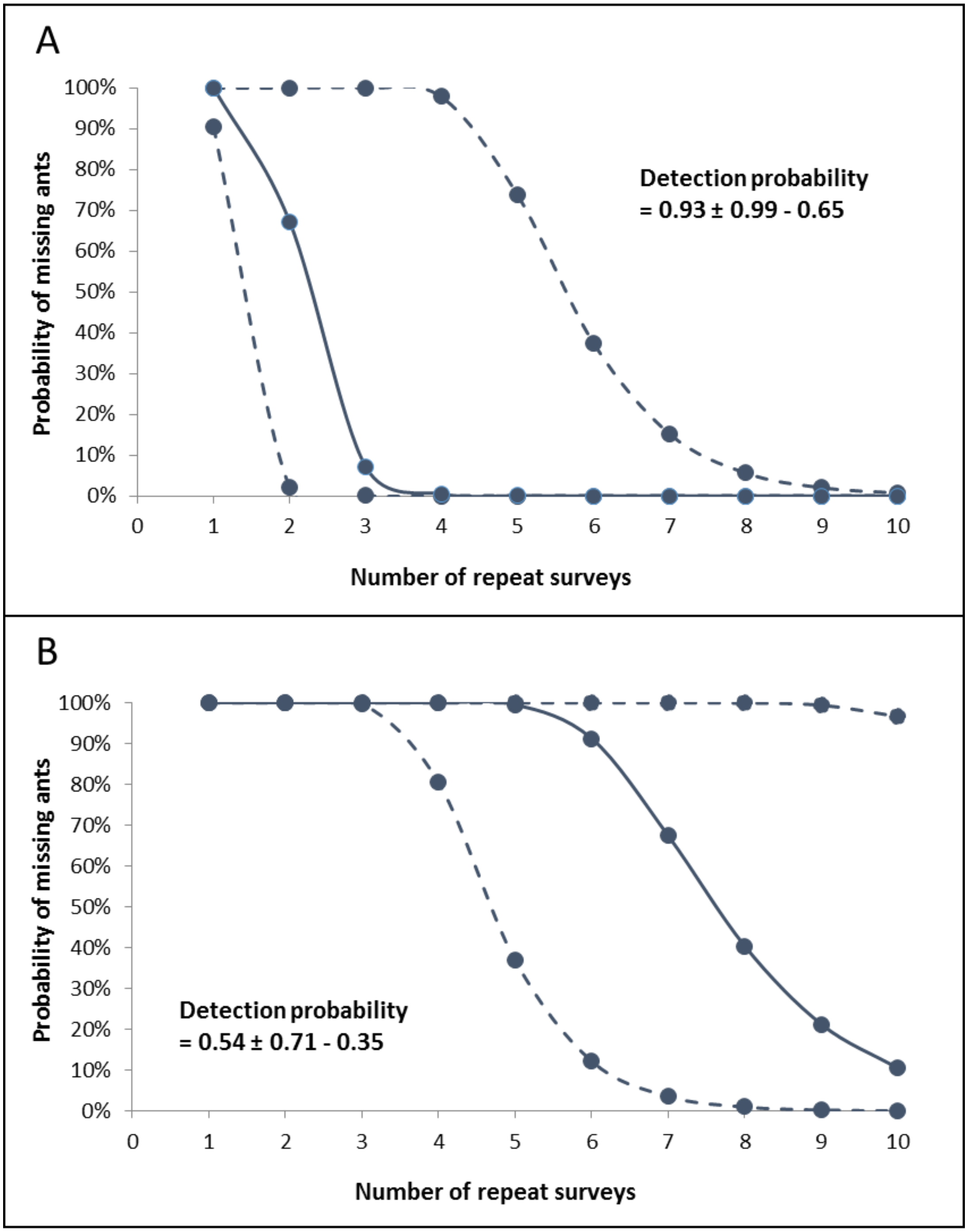

Figure 6. Cumulative probability of failing to detect YCA when present on 50 x 50 m cells by hand searching (A) and using bait tiles (B). This scenario represents 250 cells, or approximately the area north of the runway. The probabilities are cumulative and decrease with increasing number of repeat surveys. Detection probabilities (95% CI indicated by dashed lines) for the increasing number of repeat surveys are indicated for each survey method.

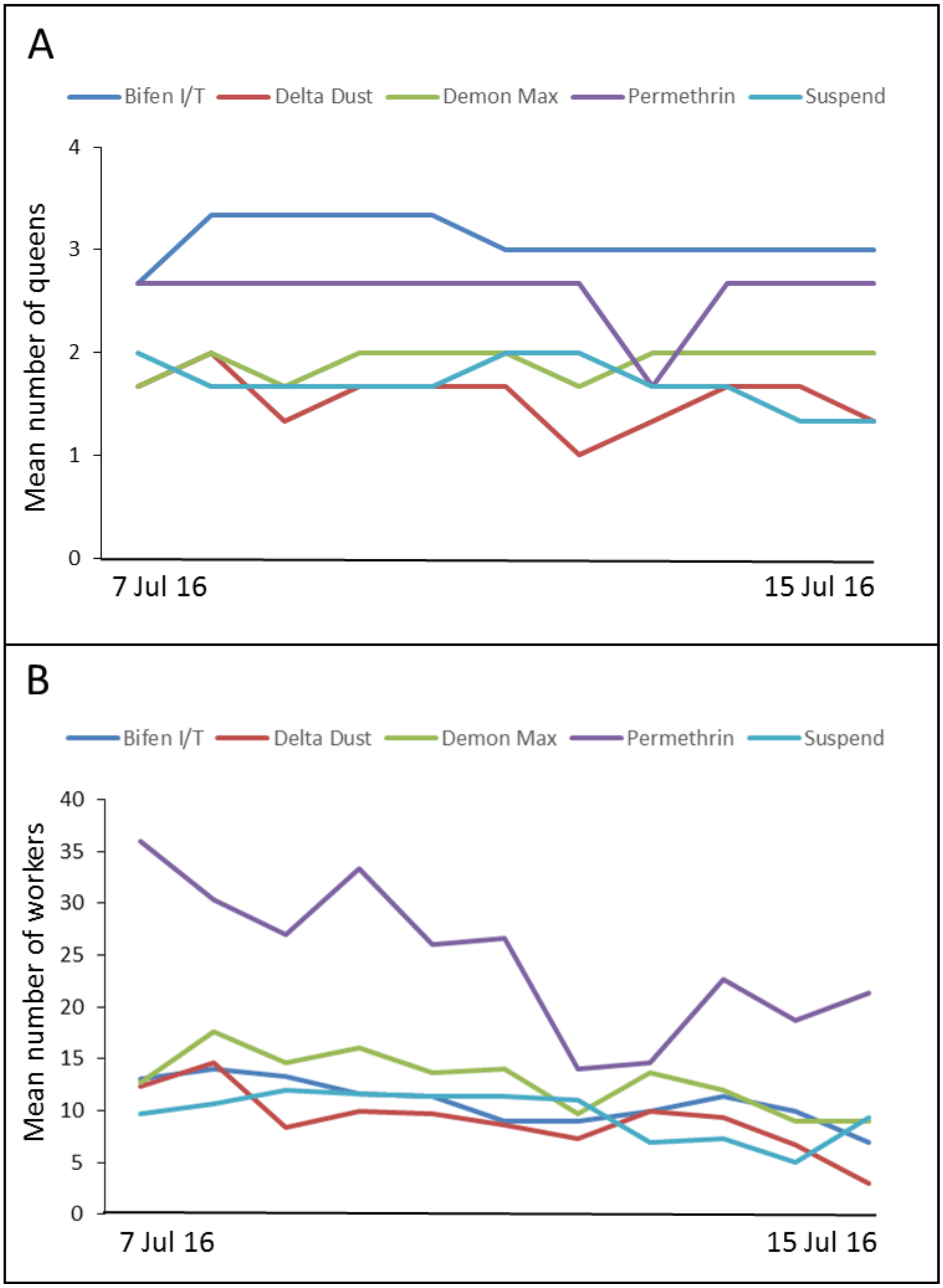

Figure 7. Survival rates of YCA queens (A) and workers (B) exposed to five types of contact insecticides mixed in soil and placed into tubes that connect nests to feeding arenas.

Discussion

Results from CAST X–XII showed that hydrogel crystals saturated with a sucrose solution containing the insecticide dinotefuran used alone, or in combination with cat food bait, is highly effective at killing YCA at the landscape level on Johnston Atoll. Between June 2015 and December 2016 (spanning deployment of CASTs X–XII), YCA had been reduced from being widespread and abundant (i.e., occupying approximately 46% of 623 bait stations within the core of the infestation) to becoming rare and difficult to detect. In fact, no YCA were detected at more than 2,300 bait stations surveyed during the last two 50-m and treatment monitoring events conducted during Oct–Nov 2016. Additionally, of the sixty 50 x 50 m cells that had been targeted for intensive treatment and monitoring during 2016, none were known to support active nests at the end of CAST XII. Nevertheless, given the difficulty of detecting YCA at very low densities, it seems likely that a few small YCA nests persist and that additional surveys and treatments will be needed to achieve eradication.

Hydrogel had a strong, immediate impact on YCA when applied at the zone level. Following the first treatment, the number of stations at which YCA were found on quadrats was reduced 87.9% and the total number of YCA detected fell 97.7%. These results parallel the outcome found on a smaller scale during CAST IX, when YCA abundance was reduced >98% 180 hours after the application of similar hydrogel bait on 50 x 50 m plots (Peck et al. 2016). Our results also showed hydrogel to be more effective than cat food bait after one application, as cat food bait reduced the frequency of detections and abundance of ants by only 63.7 and 81.2%, respectively. Hydrogel also proved effective when it followed cat food in the treatment strategy, as it further reduced YCA detections to <4% of their original abundance on four of the five zones on which it was applied. Overall, about 10% of YCA remained in one zone after the second treatment, but many of these ants were found during a single quadrat assessment that encountered over 40 ants.

The overall effectiveness of hydrogel bait at the landscape level was clear, but in several cases YCA nests survived numerous treatments of both hydrogel and cat food bait. During targeted treatment of 65 different cells, YCA were eradicated (as determined by four observation periods without ants being detected) from 55 cells (84.6%) after three or fewer applications of bait (90.9% of the applications were hydrogel). In contrast, 10 cells (15.4%) required 4–7 applications of bait (all were hydrogel) before YCA were no longer detectable. It is unclear why some nests survived long after others had died, but the persistent nests generally shared the feature of being located in dead plant tissue, among the roots of large trees, or under slabs of concrete. Microhabitat characteristics that may explain this pattern were not measured, but these substrates likely provided conditions favorable to supporting relatively large numbers of queens that produced prodigious numbers of brood. Features that may harbor large nests include trees that support an extensive and dense canopy and deep roots, a high input of organic material that supports high densities of arthropod prey, and flowers and fruit that provide a rich source of carbohydrates. Furthermore, it is possible that the availability of plant carbohydrates may have rendered the hydrogel bait less attractive to YCA, reducing its effectiveness. As a result of this potential source of competition, considerable effort went into removing flowers, particularly from kou and sea grape, prior to treatment of hydrogel during targeted treatment. In contrast to these persistent cases, nests that were found at the base of kiawe, Indian fleabane and other small trees were generally extinguished with few treatments.

Overall, we found little evidence suggesting that YCA developed an aversion to the hydrogel bait. During zone-wide applications of hydrogel, bait was applied at about 2–4 week intervals, and both ant detections and abundances generally decreased toward zero with each successive treatment. A similar pattern was found using sucrose-based hydrogel bait against Argentine ants on Santa Cruz Island, California, where ants continued to decrease in abundance and were largely eradicated in two approximately 4 ha treatment areas following four monthly applications of bait (Boser et al. 2014). The highly attractive nature of sucrose to many ant species, combined with the ant's inability to detect the insecticides at low, but lethal concentrations, likely contributed to the overall success of the bait.

The inclusion of HST surveys (hand search and bait tiles) into the YCA searching protocols during CAST XI greatly increased the likelihood that ants would be detected once they had been reduced to very low densities by zone-wide treatments. Hand searching, in particular, was effective as it produced 83% more detections than were obtained using a large number stations using Spam bait (12 stations per 50-m cell). In contrast, bait tiles increased the overall detectability of YCA in these cells by <5%. Although the two methods used in concert were slightly more effective than hand searching alone, the cost, in terms of person-hours available for searching, proved prohibitive, so CAST XII suspended the use of bait stations during intensive hand searching efforts. Hand searching, combined with monthly 50–m and treatment monitoring surveys, was a productive approach to finding surviving YCA.

Contact insecticides showed much promise for eradicating nests that may be difficult to eliminate using ingestible toxic bait. Although the number of experimental trials was low, each of the drench and dust insecticides killed worker ants in less than 15 minutes after direct exposure. The challenge of using these insecticides to eradicate nests in the field would be to ensure that queens and their developing brood come into contact with the toxin. Nests located among the roots of trees are probably most amendable to treatment with contact insecticides. The soil harboring nests could be thoroughly bathed in liquid insecticide, soaking its inhabitants. Where the precise location of a nest is not known, it would be necessary to drench a relatively large area to ensure treating the nest. The volume of solution needed to percolate into the soil to a depth at which nests would be expected to be found would vary with substrate type so the development of application protocols would require testing. It is not known whether YCA residing in the pupal stage would be affected by the insecticide drenching the nest, but these ants would likely still die upon emergence if the toxin had not been washed from the soil by rain.

Dust insecticides may kill YCA that nest in or under substrates that are impermeable to liquid drench, such as in branch cavities or under concrete structures. In these cases, the dust could be effective if blown into passageways that lead to the nests. The lab study conducted by CAST XII suggests that workers returning to the nest may carry insufficient insecticide to kill queens, but the toxin should kill workers that pass through it. Nests stressed by worker death then may lead queens to relocate, directly exposing them to the insecticide as they exit the nest. Although likely less effective than nest drenching, dust insecticides may be an important complement to hydrogel bait for eradicating nests in some protected locations.

Confirming eradication of YCA

Knowing the probability that YCA are detected when present during a survey provides a powerful tool for estimating a level of confidence that ants have been eradicated from an area. While this metric does not guarantee that eradication has occurred, it does provide managers with a quantitative measurement that can be used to decide how to allocate search-related resources. Based on ant abundances present during HST surveys, we determined that three independent searches of an area conducted by hand that detected no YCA provide a 93% confidence level that YCA were truly absent from that area. That is, YCA would be expected to be missed in 7 out of 100 surveys. However, a fourth search of the area by hand without finding ants would increase the level of confidence that ants were absent to 99%. In contrast to hand searching, ten repeated surveys using the high density array of bait stations (12 stations per cell) would be required to attain 90% certainty that YCA were not present.

These results are based on the detectability of YCA in the 160 cells surveyed during Jan–Mar 2016. Several factors of unknown influence may affect YCA detectability over space and time. These include overall ant abundance, activity level, and behavior (e.g. foraging at flowers where they may be conspicuous) as well as the structure of the habitat that may impact the efficiency at which the area can be searched. At the time of the HST surveys, the entire infestation had already been treated with toxic bait (primarily hydrogel) on three occasions and surviving YCA nests were widely distributed and worker abundances suppressed to low levels. In addition, much effort before the surveys had been made by CAST XI to clear vegetation that would allow the cells to be thoroughly searched for ants. Therefore, YCA detectability estimated at that time was likely similar to what would be expected to occur during future surveys.

Our estimate of detection probability was based on surveys of 50 x 50 m cells, but the estimate also applies to other spatial scales as long as the efficiency of the search is not influenced by the amount of area surveyed. An example of a potential compromise to the integrity of the estimate would be if the search area was too large to ensure that coverage was complete or if the focus of searchers could not be maintained over the duration of the search. In those situations, the detection probability would be lower than estimated for 50 x 50 m cells.

Few YCA have been detected beyond the infestation area during the past few years. In cases where YCA were found outside the infestation area (e.g. near camp, about 530 m west of zone 1) the ants were eradicated using a variety of insecticidal baits. While these detections have been addressed, it is possible that YCA currently exist elsewhere on the island. Island-wide bait surveys conducted twice per year on the 50-m grid have not detected YCA since 2010. Although these surveys are not as powerful as hand searching at detecting YCA, they do suggest that YCA have primarily been confined to the area delimited by the infestation boundary.

Summary

Hydrogel crystals saturated with a sucrose solution containing the insecticide dinotefuran was found to be highly effective at eliminating YCA when applied across broad sections of the 57 ha infestation area. Three applications of hydrogel alone or in combination with cat food bait, across the infestation reduced abundances of this ant by 98%. Subsequently, an intensive survey effort that utilized hand searching and a high density of bait stations (HST protocols) was used to detect surviving nests that were often small and isolated from each other. Once YCA were found, one or more bait treatments focusing on individual 50x50 m cells harboring the ants were applied until ants were no longer found. By December 2016, YCA were rarely detected during 50 m and treatment monitoring surveys. An analysis of HST data to estimate the probability that YCA are detected in an area when present revealed that four hand searching surveys using HST protocols, during which no YCA are found, are required to ensure with 99% probability that the ants have been eradicated from that area. Eradication of YCA on Johnston Atoll is increasingly attainable and may be confirmed with the continuation of intensive surveys with high detection probability and focused treatment and monitoring of areas in which the ants are found.

Suggestions for future work on Johnston Atoll

Collectively, CAST X–XII were successful at detecting and treating YCA, and have reduced the number of surviving ants to a point where they are now difficult to find. At this point, the primary challenges for CAST XIII are to locate and kill surviving nests and to conduct surveys that will contribute to attaining sufficient levels of confidence that YCA have been eradicated from the island. To meet these challenges, CAST XIII may consider:

- Continue to conduct hand searching surveys following HST protocols across all zones of the infestation.
- Intensify treatment and monitoring efforts when YCA are found.
- Use contact insecticides when feasible to kill nests. Drenching nests located in the soil with liquid insecticide would likely be very effective.
- Continue to conduct Spam-based monitoring at monthly intervals across the infestation.
- Treat the entire infestation with hydrogel at least once to eliminate nests that may have been missed during hand searching and bait monitoring.
- Increase the intensity of searches conducted outside of the infestation area.

It is only with great caution that eradication of a pest species can be declared in an area that it once occupied. It has been suggested that two years of assessment without detecting targeted ant species may be adequate to consider eradication achieved (Hoffmann and O'Connor 2004), but the assessment effort should be considered when evaluating this condition. A high degree of confidence that YCA have been extirpated would be 99%, which can be reached when YCA have not been detected in four consecutive hand searching surveys. A potentially cost-effective way to ensure this level of confidence across the entire infestation is to systematically eliminate areas that have been demonstrated to be free of ants. For example, zones 1 and 9 and all cells south of the runway are areas in which ants have been least frequently detected. If searches (past and future) indicate that ants no longer exist in these areas then they can be removed from the search queue. Zones 2 and 8 may then follow this pattern, further reducing the area over which resources need to be allocated.

The likelihood of eradicating YCA from an area in which it has been detected is enhanced when a nest can be located because it allows both treatment and follow-up monitoring to be more focused. Knowing where a nest is located also may open the opportunity for a contact insecticide, such as a soil drench, to be used. Therefore, once YCA are detected, locating the nest, or nests, that are active in that area will greatly facilitate eradication. It is important to consider that once a nest is stressed, either through bait treatment or disturbance to the substrate, surviving queens may move to a new location. As a result, post-treatment monitoring should encompass an area beyond the initial nest site. The distance that queens may move is unknown but careful searching within 20 m of a treated nest should substantially improve the odds of detection. Current protocols state that four post-treatment surveys spaced a minimum of one week apart be conducted before declaring the area free of ants. However, because some nests have proven very difficult to kill and because it is increasingly difficult to find nests as they become rarer, increasing the number and intensity of searches following treatment may be warranted.

It is important to consider that YCA may currently exist outside the infestation area. While island-wide 50-m surveys have failed to detect YCA over the past six years, additional search efforts would increase overall confidence that the ants are not present. This is particularly challenging because the area encompasses about 180 ha. Regardless, an effort that at minimum focuses on locating and searching 1) cells that are adjacent to the infestation area, and 2) habitats that YCA are known to prefer may increase the chance of detecting the ants in this area. Hand searching all cells that border the outer perimeter of the nine treatment zones at least one time is recommended. In addition, locating and searching all trees and shrubs that may provide a rich source of carbohydrates, such as sea grapes and kou is important. Finally, if resources are available, hand searching a subset of randomly chosen cells located outside the infestation area for YCA would provide additional confidence that this ant does not exist in this area of the island.

Even if Johnston is considered free of YCA during CAST XIII, a prudent long-term strategy for the island may include continued monitoring coupled with an occasional infestation-wide treatment using hydrogel bait to prevent any surviving nests from expanding and to stop new invasions before they become widely distributed. Although not as effective as hand searching, Spam-based monitoring is a relatively efficient way to survey a large area, continued monitoring at monthly intervals would likely detect YCA if they were present. Similarly, treating the entire infestation area with hydrogel bait one or two times every six months would help ensure that surviving undetected nests are killed.

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