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EFFORTS TO ERADICATE YELLOW CRAZY ANTS ON JOHNSTON ATOLL: RESULTS FROM CRAZY ANT STRIKE TEAM IX, DECEMBER 2014-JUNE 2015

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Abstract

The ecologically destructive yellow crazy ant (YCA; *Anoplolepis gracilipes*) was first detected on Johnston Atoll in January 2010. Within eight months, the U.S. Fish and Wildlife Service had mobilized its first crazy ant strike team (CAST), a group of biologists dedicated to testing and identifying insecticidal baits to be used to eradicate the ant on the atoll. During December 2014–May 2015 CAST IX focused on testing hydrogel crystals saturated with sucrose solution (25%) carrying the insecticides thiamethoxam and dinotefuran against YCA. A series of experiments, including artificial nest box trials, and field-based palatability trials and eradication tests on small (500 m^2 or 0.05 ha) and large plots (2500 m^2 or 0.25 ha), were conducted to test concentrations of thiamethoxam ranging from 0.0005% to 0.01%, and dinotefuran at 0.05%. Additionally, the cat food-based matrix containing dinotefuran (0.05%), the standard bait used to suppress YCA on Johnston since 2011, and textured vegetable protein (TVP) carrying dinotefuran at 0.1% and 0.05% were included in large plot tests. Nest box trials were inconclusive due to a consistent loss of queen and worker ants in control boxes, so they were discontinued. Palatability trials suggested higher dosages of thiamethoxam (0.005 and 0.01%) were less attractive than lower dosages (0.0005 and 0.001%) and controls (sucrose only), but small and large plot experiments failed to identify a thiamethoxam concentration that was consistently effective at killing YCA. In contrast, hydrogel containing dinotefuran was consistently effective, killing >95% of YCA on small and large plots. As expected, the cat food bait effectively reduced YCA abundances, but was slightly less effective than hydrogel containing dinotefuran over time. Three successive, approximately weekly treatments of large plots with hydrogel bait, or other baits followed by hydrogel bait, suggest an increasing overall effectiveness, with no aversion of YCA to the bait. This finding is important in that it indicates that hydrogel bait can be applied at short time intervals, potentially resulting in relatively constant exposure of YCA to highly attractive, yet toxic, sucrose-based bait. TVP performed similar to hydrogel, reducing YCA abundance >92% at both concentrations tested. Finally, dosages of hydrogel containing dinotefuran at 6, 12 and 24 l/0.25 ha were all effective at reducing YCA on large plots. Overall, results from these experiments suggest that hydrogel containing dinotefuran (0.05%) is a promising tool for eradicating YCA on Johnston Atoll.

Introduction

The yellow crazy ant (YCA; *Anoplolepis gracilipes*) was first detected on Johnston Atoll in January 2010. At that time, the infestation occupied about 38 ha and the ants were having strong negative impacts on ground-nesting seabirds, largely displacing the birds from the infestation area. The U.S. Fish and Wildlife Service recognized the severity of the problem and quickly formed and mobilized the first Crazy Ant Strike Team (CAST). By August 2010, this small group of biologists was stationed on the atoll and charged with developing methods to eradicate YCA. During this process, several commercial baits that showed promise during trials against YCA in Hawaiʻi were tested on Johnston, but they proved ineffective at killing YCA in experimental plots. The development and application of custom bait containing the active ingredient dinotefuran (Safari® SG Insecticide) during 2011–2012 resulted in a dramatic reduction in YCA abundance on Johnston and effectively limited the spread of the ant on the atoll. While reducing YCA abundance >90% in most instances, this custom toxic bait failed to eradicate the ants, necessitating the development of alternative baits.

Recent success of a novel bait matrix utilizing dehydrated polyacrylamide polymers ("hydrogel crystals") against Argentine ants (*Linepithema humile*) on the Channel Islands off the coast of south-central California (Boser *et al*. 2014) led to efficacy testing of this matrix on Johnston Atoll. Hydrogel bait is formed by adding a water-soluble insecticide to a sucrose solution that is used to rehydrate the hydrogel crystals (hereafter referred to as hydrogel). Testing of different concentrations of insecticide against ants in lab and field trials is essential to identifying amounts that are toxic to queen and worker ants yet do not deter foragers. Testing of the neonicotinoid insecticide thiamethoxam in hydrogel containing sucrose against Argentine ants revealed an effective concentration of 0.0006% (Boser *et al.* 2014) to 0.007% (Buczkowski *et al.* 2014a, b). This insecticide and concentration was the starting point for trials conducted by CAST IX against YCA on Johnston Atoll during December 2014—May 2015.

The primary objective of CAST IX was to conduct lab and field trials to test the efficacy of hydrogel bait saturated with a sucrose solution (25%) containing the insecticide thiamethoxam against YCA. As the work progressed, the objectives shifted toward additionally testing of the insecticide dinotefuran in the hydrogel matrix. Fundamental to meeting these objectives was to identify the concentrations of thiamethoxam and dinotefuran that were most effective at killing queen and worker ants, as well as the most effective rate at which these baits should be applied over the landscape. It was planned that CAST IX would develop the information needed for CAST X to attempt an island-wide eradication of YCA using hydrogel-based bait.

METHODS

Bait matrices

Hydrogel bait is comprised of dehydrated hydrogel crystals (Miracle-Gro® Water Storing Crystals, The Scotts Company, Marysville, OH) saturated with a 25% solution of sucrose (250 g sugar mixed into 1000 ml of water) into which water-soluble insecticide has been added (Figure 1). Insecticides tested included thiamethoxam (Optigard® Flex liquid containing 21.6% thiamethoxam; Syngenta Crop Protection, Greensboro, NC) and dinotefuran (Safari 20 SG Insecticide pellets containing 20% dinotefuran; Valent Corporation, Walnut Creek, CA). Table 1 shows the amount of thiamethoxam stock solution (0.0216% thiamethoxam) needed to prepare several different concentrations of hydrogel bait.

The following formula was used to calculate the thiamethoxam concentrations presented in Table 1:

$$c_1 * v_1 = c_2 * v_2$$

where c_1=initial concentration of thiamethoxam (%), v_1=unknown volume of thiamethoxam needed (ml), c_2=desired concentration of thiamethoxam (%), and v_2=volume of the final solution (ml). For example, to make a 0.0005% solution of thiamethoxam (from a stock solution at 0.0216%), add 23.15 ml to 1000 ml water ($0.0216 * v_1 = 0.0005 * 1000$).

Hydrogel bait containing dinotefuran is made in a manner similar to thiamethoxam except the dry Safari pellets are dissolved in water at a rate of 2.5 g/l to make a 0.05% solution. Alternative concentrations can be made using the formula above.

Figure 1. Hydrogel crystals before (left) and after (right) saturation with a sucrose (25%) solution. Twenty grams of dry crystals expands to make approximately one liter of bait.

Table 1. Amount of thiamethoxam stock solution (in ml) needed to prepare several desired concentrations of hydrogel bait. The stock solution is 0.0216% thiamethoxam (1 ml Optigard Flex mixed in 1000 ml water). Amounts of stock solution needed to prepare four different volumes (100 to 1000 ml) are shown.

Desired concentration		Stock solution needed (ml)			
%	ppm	1000 ml	500 ml	250 ml	100 ml
0.0005	5	23.15	11.57	5.79	2.31
0.001	10	46.30	23.15	11.57	4.63
0.002	20	92.59	46.30	23.15	9.26
0.005	50	231.48	115.74	57.87	23.15
0.01	100	462.96	231.48	115.74	46.30

The standard cat food bait containing dinotefuran at 0.05% used to suppress YCA abundances on Johnston since 2011 was compared to hydrogel bait in some trials. The recipe to make 1 kg of cat food bait includes: 250 g canned cat food (Friskies®, Nestlé Purina, St. Louis, MO), 250 g of corn-based Karo® dark syrup (ACH Food Companies, Inc., Cordova, TN), 7 g powdered xanthan gum, 500 ml water, and 2.5 g Safari 20 SG Insecticide. This paste-like bait is applied using a high-volume squirt gun.

Textured vegetable protein (TVP; Honeyville®, Brigham City, UT) was also tested as an alternative to hydrogel as a carrier for the insecticides in some trials. Similar to hydrogel, TVP is produced as a dehydrated medium and rehydrated with the sucrose solution containing insecticide. The method for preparing the insecticide solution used with TVP was identical to that used for hydrogel.

Palatability trials

Palatability trials were conducted to assess the relative attractiveness of different concentrations of thiamethoxam incorporated into the hydrogel matrix. Palatability trials are essentially "taste tests" during which ant foraging behavior is compared between control baits (hydrogel containing just 25% sucrose solution) and a series of baits containing progressively greater concentrations of thiamethoxam. Because palatability trials indicate preference among food options, they only suggest how baits may perform in the field; field trials are needed to verify the performance of the bait at killing ants. Thiamethoxam was tested in palatability trials at the following concentrations (%): 0 (control); 0.0005; 0.0001; 0.005; 0.01. This range encompasses the concentrations found to be effective against Argentine ants (Buczkowski *et al.* 2014a, b., Boser *et al.* 2015). The bait was prepared following the procedure outlined in Appendix 1 and the concentration determined using the values provided in Table 1.

Four sites containing relatively high densities of YCA and located at least 50 m apart were identified for testing. Because YCA nests are most commonly found within the root structure of trees (e.g., sea grape; *Coccoloba uvifera*), test sites were located near trees. At each site, the different concentrations of hydrogel were presented to YCA in a series of arrays, with each array representing each of the five bait types. Within each array, 8–10 crystals of each bait type were placed on plastic cards located approximately 10 cm apart and about 2 m from the base of the tree (Figure 2); this array was repeated four times with arrays placed at least 5 m apart within each site (4 arrays x 5 bait types = 20 cards/site).

Palatability assessments were based on 1) the number of ants on each card at 10, 30 and 60 min after bait placement on the ground, and 2) the amount of time individual ants spent feeding on the bait. The second measurement was based on the activity of 15 sequentially chosen ants at a subset of bait cards at each thiamethoxam concentration. Timed feeding observations were conducted within one hour of bait placement on two of the four study plots. Timed feeding observations were terminated at 300 seconds (5 min). To minimize the effect of desiccation on hydrogel bait, the bait was prepared immediately prior to the experiment and trials were conducted early in the morning. Two trials were conducted each on 30 December 2014 and 6 January 2015.

The relative attractiveness of hydrogel containing different concentrations of thiamethoxam was evaluated using one-way ANOVA following $\log_{10}$ transformation of counts of YCA at cards (SYSTAT 13; SYSTAT Software, Inc., Chicago, ILL). Subsequent to a finding of overall significance ($P < 0.05$), individual pairwise comparisons among each concentration were made using a Tukey's Honestly-Significant-Difference Test.

Figure 2. Array of hydrogel baits of differing in concentration of thiamethoxam placed on plastic cards used in palatability trials.

Trials testing thiamethoxam against YCA colonies in artificial nest containers

Trials using small YCA colonies placed in artificial nests were conducted to evaluate the efficacy of thiamethoxam at different concentrations on the survival of worker and queen ants. Artificial nests were constructed by pouring liquid plaster-of-Paris into 2.25-l plastic sandwich containers (Figure 3). Three or four cavities for nests were created in each container by temporarily placing modeling clay approximately 2 cm thick onto the surface of the plaster before it solidified to create a void in the matrix. A glass plate was gently embedded on top of the matrix before curing to allow the nest cavities to be viewed and ants counted. When not being viewed, nests were covered with aluminum foil to maintain darkness. Feeding arenas, consisting of containers similar in size to nest boxes, were used to supply bait to YCA colonies. Nest containers were linked to feeding arenas via 1/2 inch diameter flexible tubing that allowed foraging worker ants to feed on experimental bait. For each nest, 4–5 queens (mean = 4.6) and approximately 120 workers (mean = 117.8) were collected from nests in the field, added to artificial nests within 1–2 h, and allowed to acclimate, with water but without food, for 48 h. After this time, water was removed and hydrogel added to the feeding arenas where it remained available to foraging workers for 24 h. Bait toxicity was assessed by estimating the number of dead workers within nests or feeding arenas daily for seven days. Thiamethoxam was tested at the following concentrations (%): 0 (control); 0.0005; 0.0.001; 0.005; 0.01. The palatability trials were repeated one time using newly acquired queen and worker ants. In addition to the hydrogel, textured vegetable protein (TVP) was tested as an alternative carrier of thiamethoxam on one occasion using thiamethoxam concentrations identical to that used in hydrogel trials.

Figure 3. Artificial nest boxes used to test the efficacy of thiamethoxam differing in concentration on survival of queen and worker ants. The photo on the left shows the feeding arena (with the blue lid) attached via flexible tubing to the nest box (covered with aluminum foil). The photo on the right shows the nest box containing ants within linked chambers. A hole leading to the flexible tube is visible at the top of the uppermost chamber. A removable glass plate embedded in the plaster matrix allows inhabitants of the nest, including dead ants, to be seen.

Small plot field trials testing hydrogel bait against YCA

Small scale field trials were conducted to evaluate the efficacy of hydrogel as a carrier for thiamethoxam and dinotefuran. Similar to the palatability trials, test plots were based around trees that supported YCA nests. Twelve tree-centered sites, each at least 50 m apart and supporting moderately high numbers of YCA, were identified for testing the bait. Around each tree a 12.6 m radius treatment area (500 m^2 or 0.05 ha) was established (Figure 4). Hydrogel bait was broadcast by hand as evenly as possible over each plot (Figure 5) during two treatment rounds at the rate of 93.6 l/ha (or 4.7 l/plot), a rate found to be effective at controlling Argentine ants on the Channel Islands (Christie Boser, personal communication). The first round tested hydrogel containing thiamethoxam at 0.0005%, 0.001%, 0.005% and 0.001%, and dinotefuran at 0.05%, against a control plot containing only sucrose (25%). Based on results from the first round, the second round of treatment was modified to test hydrogel containing thiamethoxam at 0.005% and 0.001% and dinotefuran at 0.05%. In addition to these baits, the effectiveness of dinotefuran (at 0.05%) in a cat food matrix was tested.

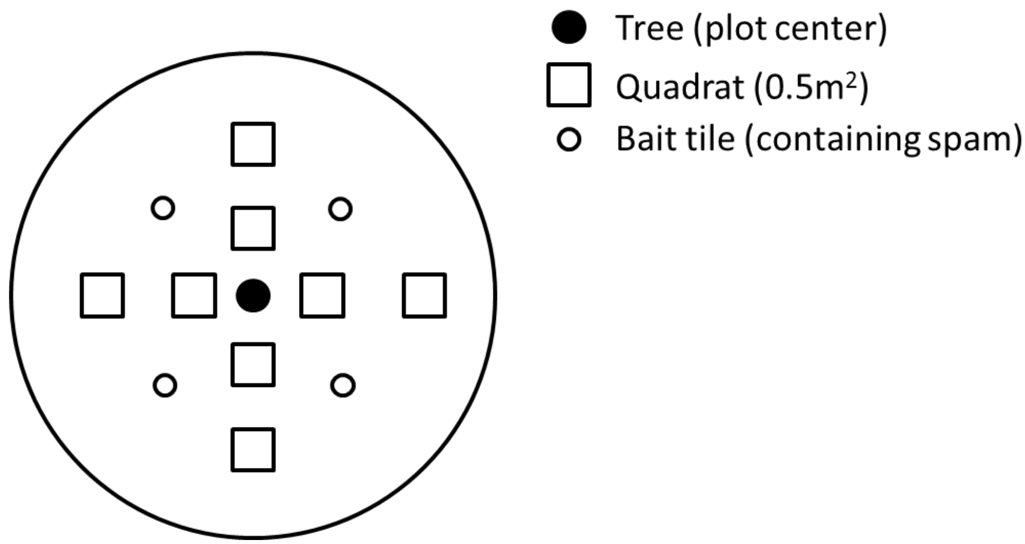


Figure 4. Schematic view of a small, tree-centered treatment plot. Each plot has a radius of 12.6 m creating a treatment area of 500 m^2. Quadrats are located 2 m (quadrats A) and 5 m (quadrats B) from the plot center while bait tiles are placed 3 m from the plot center.

Figure 5. Hydrogel bait prepared to be hand-broadcast over the study plots.

Bait effectiveness was evaluated using two complementary methods: 1) counting the number of ants at ceramic bait tiles (approximately 10 x 10 cm) containing 15 ml of spam® mixed with water (50% dilution by weight; the standard YCA monitoring bait on Johnston) and 2) counting ants within 0.5 m^2 sampling quadrats. In each plot, bait tiles and quadrats were placed in a fixed array centered on trees; four tiles were placed 3 m distant from the tree and four quadrats were placed each 2 and 5 m distant from the tree (Figure 4). Ants were counted on spam-baited tiles after 30 min of exposure. In quadrats, all YCA entering the quadrat during 30 s intervals were counted. The count was repeated after one minute, and the average number of ants detected during the two 30 s counts was used for the analysis. Ant abundances were assessed at the following times: prior to bait application (0 hr), and 12, 36, 60 and 108 hr following treatment. The first round of treatment took place on 18 February 2015 and the second round began on 16 March 2015.

Large plot field trials testing hydrogel and TVP bait against YCA

Large scale field trials testing the efficacy of thiamethoxam and dinotefuran were conducted in 50 x 50 m (2500 m^2 or 0.25 ha) plots located in areas containing moderate to high YCA abundance. In addition to testing the effectiveness of single applications of bait, this set of trials was further designed to test the effectiveness of applying baits multiple times on the same plots over short time intervals, using either the same bait, or different baits. Baits that were most effective in the small plot trials were included in the larger-plot trials, and included hydrogel containing thiamethoxam at 0.01% and dinotefuran at 0.05%. As in the small plot trials, the cat food matrix carrying dinotefuran at 0.05% was included as a standard to which the hydrogel baits could be compared. In addition to the hydrogel baits, textured vegetable protein (TVP) carrying dinotefuran at 0.1% and 0.05% applied at a rate of 6 l/0.25 ha was tested one time each, during the second and third rounds of treatment, respectively. Cat food bait was applied at the standard rate of 6 l/0.25 ha, and hydrogel was applied at the rate of 24 l/0.25 ha, except on one occasion during Round 3 when hydrogel was applied at a reduced rate of 6 l/0.25 ha. The dates and sequence during which baits were applied to each plot is shown in Table 2. Following the short-interval treatment strategy, bait was applied in Round 2 eight days after being applied in Round 1 and bait was applied in Round 3 ten days after being applied in Round 2.

Similar to small plot trials, YCA abundances in larger plots were monitored before and after treatment by counting numbers of ants within 0.5 m^2 quadrats during two 30 s intervals, and by counting the number of ants that accumulated on tiles containing spam as lure for 30 min. The monitoring array within each plot consisted of eight quadrats and five bait tiles (Figure 6). Four quadrats were each located approximately 5 and 20 m from the plot center, and four bait cards were placed about 15 m from the center and one bait card was located at the center of the plot. Monitoring began about sunrise. Abundances of YCA were monitored in each plot approximately 60 and 132 hr following treatment. Due to the short time interval between treatments on each plot, the assessment of ant abundances at 128 hr during Rounds 1 and 2 acted as the 0 hr abundance level for Rounds 2 and 3, respectively.

Table 2. Baits tested against YCA in large field plots (0.25 ha). The dates in the first row indicate when each bait was applied on each plot. One plot acted as a control and received no bait carrier or active ingredient (AI), four plots received bait applications on three occasions, and one plot received bait once. Cat food and TVP baits were applied at the rate of 6 l/0.25 ha and hydrogel was applied at a rate of 24 l/0.25 ha except on one plot (43G) during Round 3 when it was applied at 6 l/0.25 ha.

	27-Mar-15		4-Apr-15		14-Apr-15		5-May-15	
Plot	Carrier	AI (%)	Carrier	AI (%)	Carrier	AI (%)	Carrier	AI (%)
48J	none	none	none	none	none	none		
43G	hydrogel	thiamethoxam 0.01	hydrogel	thiamethoxam 0.01	hydrogel	dinotefuran 0.05		
36J	cat food	dinotefuran 0.05	cat food	dinotefuran 0.05	cat food	dinotefuran 0.05		
41I	cat food	dinotefuran 0.05	hydrogel	dinotefuran 0.05	hydrogel	dinotefuran 0.05		
50G	hydrogel	dinotefuran 0.05	hydrogel	dinotefuran 0.05	hydrogel	dinotefuran 0.05		
37H					TVP	dinotefuran 0.10		
39H							TVP	dinotefuran 0.05

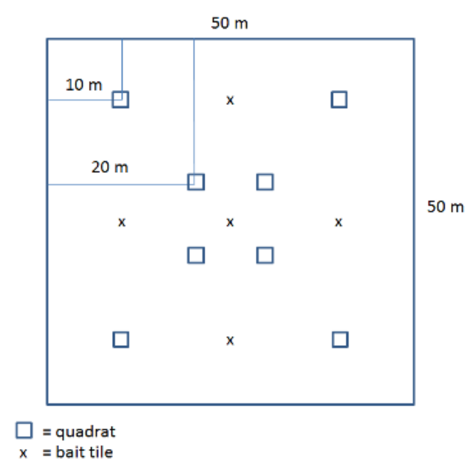


Figure 6. Schematic view of a large scale 50 x 50-m (2500 m^2 or 0.25 ha) treatment plot.

Testing hydrogel application rates on large plots

The success of hydrogel incorporated with dinotefuran (0.05%) mixed in sucrose solution (25%) led to a test to identify the minimum rate at which the bait can be applied and still maintain effective reduction of YCA. The hydrogel bait was applied at the rates of 6, 12, and 24 l/0.25 ha (based on liquid solution) in 0.25 ha (50 x 50 m) plots supporting moderate levels of YCA abundance on 19 May 2015. For comparison, a control plot of identical size was monitored. The efficacy of the application rates was assessed after approximately 84 and 180 hr using quadrats and bait tiles in a manner similar to that described previously for large plot trials.

RESULTS

Palatability trials

Thiamethoxam concentration had a significant influence on the number of YCA feeding on hydrogel crystals, particularly at the highest levels (Figure 7); compared to controls, YCA abundance was reduced 79.7% at 0.005% and 88.6% at 0.01% concentration ($P < 0.001$ in both cases). In contrast, no difference was found in numbers of foraging ants at 0.0005% and 0.001% thiamethoxam, although numbers of ants were 38.1% lower than controls at 0.001%. There was generally little difference (no accumulating effect) in YCA abundance after 30, 60, and 90 min of exposure to bait. The amount of time YCA spent feeding on hydrogel bait was also significantly influenced by thiamethoxam concentration, with mean feeding times reduced 81.2% at thiamethoxam 0.005% and 87.8% at thiamethoxam 0.01% ($P < 0.001$ in both cases; Figure 8).

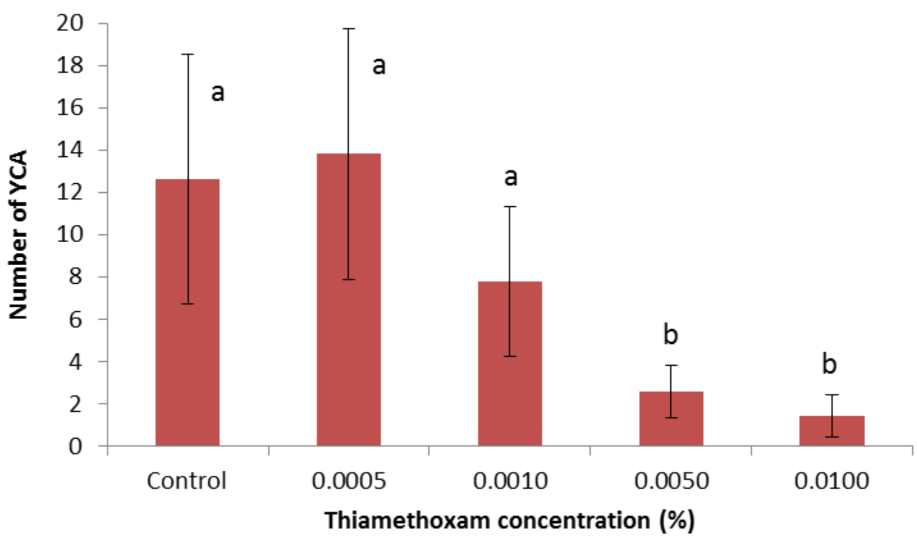


Figure 7. Mean (±SEM) number of YCA counted at bait cards on which hydrogel containing thiamethoxam of differing concentration were placed. YCA were counted after 60 min. Values are based on the four sites; within-site replicates were pooled (averaged). Concentrations sharing the same letter above the bar were not significantly different from each other.

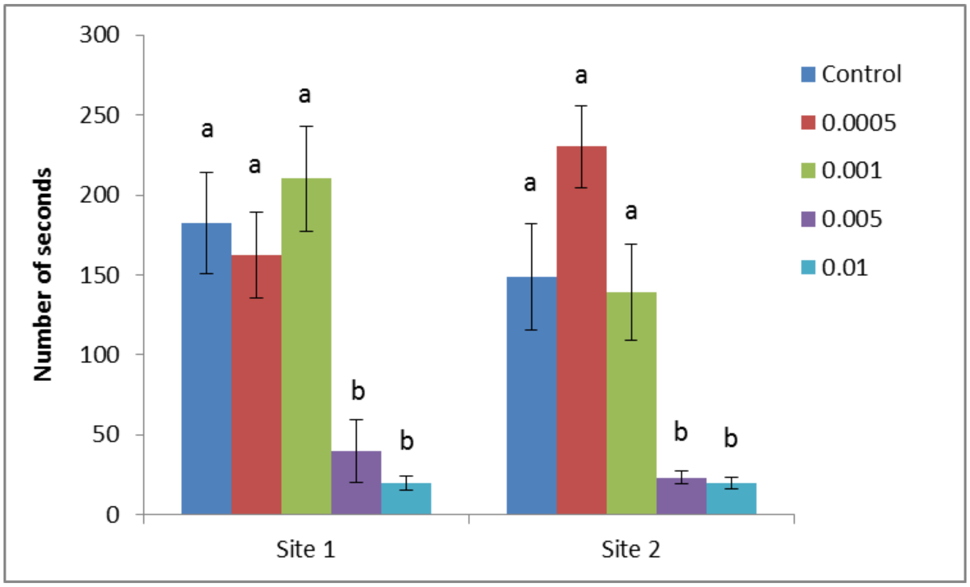


Figure 8. Mean (±SEM) duration (in seconds) that individual YCA spent feeding on bait cards on which hydrogel crystals containing thiamethoxam of differing concentrations were placed at two sites. Concentrations sharing the same letter above bar were not significantly different from each other.

Trials testing thiamethoxam against YCA colonies in artificial nest containers

Thiamethoxam toxicity trials in artificial nests could not be assessed because queen and worker ant abundances in control nests (those exposed to hydrogel containing only sucrose) could not be sustained over the duration of the study. By seven days, 23% of queens and 74% of workers had died in the four control nests. Rather than continue to invest time in nest box-based tests, effort went towards testing baits on larger field plots.

Small plot field trials testing hydrogel bait against YCA colonies

Round 1 of testing on small plots suggested that hydrogel containing thiamethoxam at 0.005% and dinotefuran at 0.05% were most effective at reducing YCA abundance (Figure 9). Quadrat-based counts of ants in plots treated with thiamethoxam at 0.005% revealed reductions of 100% and 92% in quadrats A and B, respectively, after 108 hr. Similarly, no YCA were detected in any quadrats in the plot treated with dinotefuran after 108 hr. In contrast, quadrat-based counts indicated little or no reduction in YCA abundance using thiamethoxam concentrations other than 0.005%. Bait tiles detected no ants in plots containing thiamethoxam at 0.005 and 0.01%, and dinotefuran at 0.05%, after 108 hr. Bait tiles also detected 76% fewer ants after 108 hr in the plot containing thiamethoxam at 0.001%.

Results from Round 2 diverged markedly in some respects from results in Round 1. Round 2 counts of YCA in quadrats 108 hr after treatment of thiamethoxam at 0.005% decreased little compared to pre-treatment counts, while counts in the plot treated with thiamethoxam at 0.01% decreased 49% in quadrats A and 59% in quadrats B (Figure 9). Quadrat counts in dinotefuran plots indicated an even larger reduction in YCA abundance 108 hr after treatment, with counts reduced 96% and 99% in quadrats A and B, respectively. Again, counts of YCA at bait tiles were more dramatic than in quadrats, with strong reductions found for thiamethoxam at 0.005% and dinotefuran. Few ants were detected using bait tiles prior to, or after, treatment in the thiamethoxam at 0.01% and cat food plots.

Large plot field trials testing hydrogel and TVP bait against YCA

Large plot trials revealed a weak but steady effect of thiamethoxam (0.01%) over multiple treatments, with YCA abundance reduced 26% during Round 1 and 62% during Round 2 (after 132 hr in each case on Plot 43G; Figure 10). Thiamethoxam was abandoned as a treatment at this point because it reduced YCA abundance only 71% after the two rounds of treatment. During Round 3 on the thiamethoxam plot (43G), a reduced application rate of 6 l/0.25 ha (from 24 l/0.25 ha) of dinotefuran (0.05%) in hydrogel was tested. The reduced amount of dinotefuran was ineffective in reducing ant numbers as they increased 48% by 132 hr after application. Cat food containing dinotefuran (0.05%) reduced YCA abundance 90% during Round 1 and 50% during Round 2 (Figure 10). No further reduction in ant abundance was found after the third round of cat food treatment (ants increased slightly from 0.3 to 1.0 ants/quadrat).

Dinotefuran (0.05%) incorporated into hydrogel was highly effective at reducing YCA abundance (Figures 10). YCA abundances on quadrats were reduced 96% compared to pre-treatment levels using hydrogel during Round 1. YCA abundance on the plot (50G) treated with dinotefuran in hydrogel was reduced an additional 67% following the second application and 100% after the third application (YCA abundances assessed 132 hr after the second and third applications). Hydrogel applied to the plot that received cat food during Round 1 (plot 41I) further reduced YCA abundance 69% during Round 2 and another 60% during Round 3; overall, YCA abundance was reduced 99% by the end of the third round on this plot.

Overall, results observed at bait tiles corroborated the results in quadrats, but bait tile trends were stronger, yielding no YCA following application of cat food or hydrogel during all treatment rounds (Figures 10). Ant counts at tiles were also much lower than in quadrats on the thiamethoxam plot (43G) as YCA abundance was estimated to have been reduced 95% compared to 25% at tiles and in quadrats, respectively, after 132 hr during Round 1. TVP saturated with a dinotefuran solution at 0.1% and 0.05% reduced ant abundances in quadrats 97% and 92% after 60 hr, respectively (Figure 11). And as found with plots treated with hydrogel and cat food, ant reduction at bait tiles in TVP-treated plots was greater than measured by quadrats, with no YCA found at either dinotefuran concentration after 60 hr.

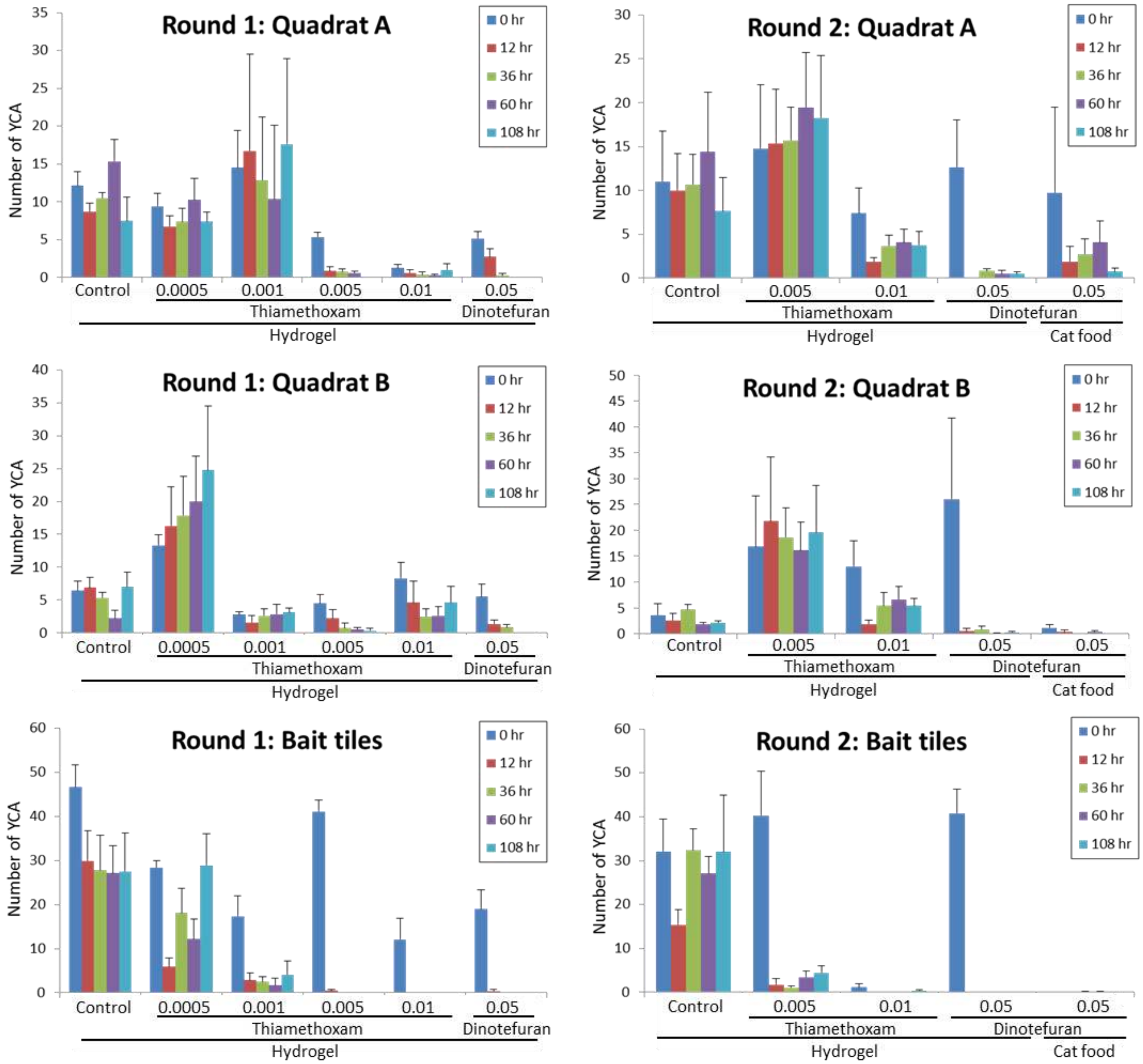
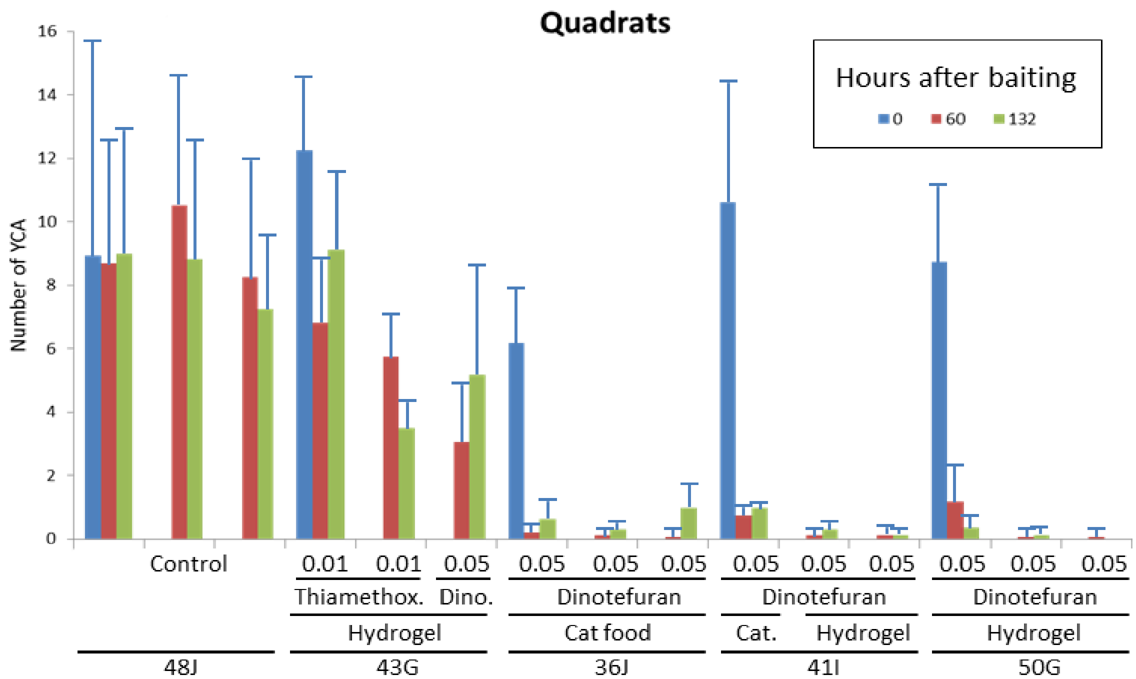


Figure 9. Mean number of YCA counted in quadrats and at bait tiles during two rounds of bait testing in small plots (500 m^2). Treatments tested during Round 1 included hydrogel containing thiamethoxam at 0.0005, 0.001, 0.005, and 0.01% and dinotefuran at 0.05%. Treatments during Round 2 included hydrogel containing thiamethoxam at 0.005 and 0.01% and dinotefuran at 0.05%, and cat food containing dinotefuran at 0.05%.



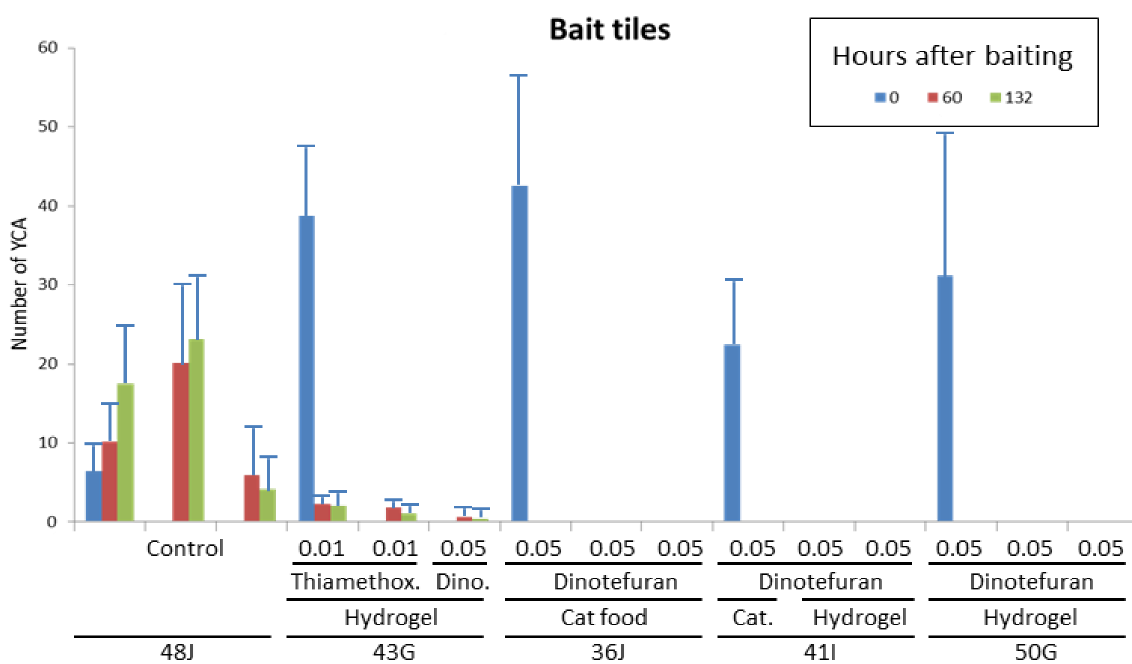


Figure 10. Number of YCA counted in quadrats and at bait tiles during three rounds of bait testing in large plots (0.25 ha). The bait carrier (hydrogel or cat food), active ingredient (thiamethoxam or dinotefuran) and concentration (0.01 or 0.05%) are nested within plots (e.g., 43G) on the x-axis. The treatment rounds are displayed from left (first) to right (third) for each plot on the top line of the x-axis. Hydrogel was applied at a rate of 24 l/0.25 ha during in all cases except during Round 3 in plot 43G when it was applied at a reduced rate of 6 l/0.25 ha. No bait was applied on the control plot. Note that on each plot the "0" hr for Rounds 2 and 3 was the "132" hr of Rounds 1 and 2, respectively.

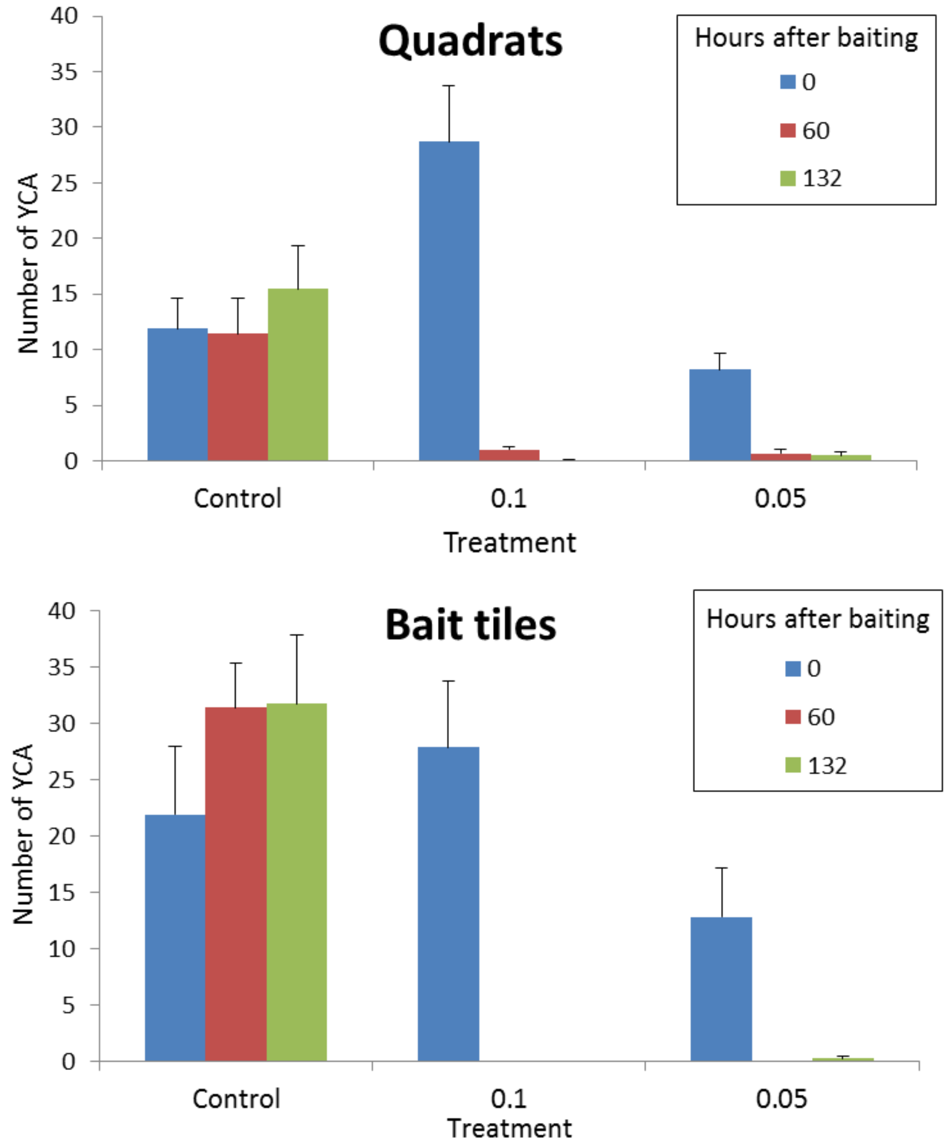


Figure 11. Mean (±SEM) number of YCA counted in quadrats and at bait tiles following application of dinotefuran incorporated into TVP at concentrations of 0.1% and 0.05% in large plots (0.25 ha). TVP was applied at the rate of 6 l/0.25 ha.

Testing hydrogel application rates on large plots

All three rates at which hydrogel containing dinotefuran (0.05%) were tested in large plots were highly effective at reducing YCA abundances. Counts of YCA in quadrats in plots receiving hydrogel at 12 and 24 l/0.25 ha decreased >98% after 180 hr, while no YCA were detected in the plot receiving hydrogel at 6 l/0.25 ha at 180 hr (Figure 12). No YCA were detected at bait tiles in any of the treatment plots following the application of hydrogel bait.

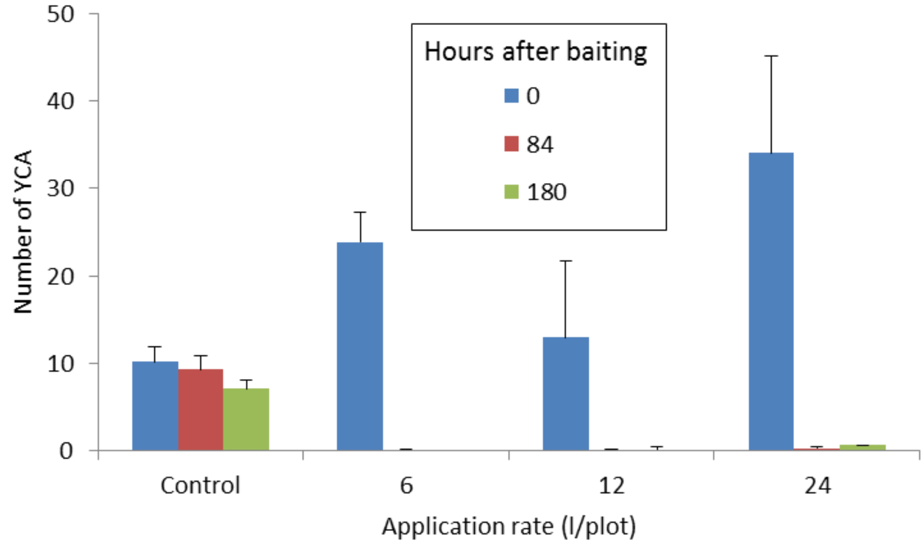


Figure 12. Mean (±SEM) number of YCA counted in quadrats on large plots (0.25 ha) following hydrogel treatment at three application rates (6, 12, and 24 l/0.25 ha) and on a control plot.

DISCUSSION

Hydrogel containing thiamethoxam dissolved in sucrose solution was relatively ineffective against YCA on Johnston Atoll, contrasting the success of similar bait against Argentine ants in the Channel Island (Boser *et al.* 2014). Palatability trials revealed YCA to feed on hydrogel containing thiamethoxam at 0.0005% and 0.001% in roughly similar abundance and duration to hydrogel containing only sucrose, but both small and large plot trials showed relatively weak efficacy in killing ants at any of thiamethoxam concentrations tested. It is not clear why thiamethoxam failed in field trials, but it is possible that additional testing may reveal a more effective concentration. However, the success of dinotefuran-based baits rendered thiamethoxam expendable for eradicating YCA on Johnston.

Dinotefuran, the active ingredient (AI) in Safari 20 SG Insecticide, was highly effective at reducing YCA abundances in field trials. Dinotefuran is the AI incorporated into the cat food matrix that has been used effectively at suppressing YCA abundance on Johnston since 2011. In both small and large plot trials, hydrogel containing dinotefuran (0.05%) reduced YCA abundances by more than 90% after one application. The promise of this bait as an eradication tool was further highlighted by the absence of YCA in a large plot on which this bait was applied three times at approximately one-week intervals. This result supports the finding on the Channel Islands that Argentine ants do not develop "bait shyness" toward the sucrose-based hydrogel after repeated, short-interval application of the bait (Boser *et al.* 2014). This powerful finding indicates that hydrogel bait could be used multiple times in succession or as an alternate after the application of bait utilizing a different matrix (e.g., cat food). The large plot trial suggests that an application of dinotefuran in hydrogel following an initial treatment of cat food was more effective than cat food alone applied in succession, further promoting the pairing of these baits as an eradiation strategy. A slight increase in YCA abundance during the third application of cat food bait on a large plot suggested that ants avoided the bait when applied in succession, possibly recognizing it as a toxicant. Unfortunately, nest box trials were not conclusive with respect to bait impacts on queen ants. Further study determining how best to maintain nests containing several queens and 100 or more workers in small boxes would likely lead to a productive evaluation of how baits work at the individual nest level, and could be a fruitful pursuit if alternative baits are to be tested further.

The hydrogel tested in this study is composed of synthetic cross-linked polyacrylamide polymers, and is most commonly marketed as a soil amendment because of its water-retaining abilities. When rehydrated with water, the crystals swell to form a gel several times their original size and provide a moist surface from which ants can imbibe the sucrose solution. At effective dosages, YCA presumably are attracted to the sucrose solution without detecting the insecticide. The gel is subject to desiccation by sun and wind, which likely decreases its effectiveness over time. However, preliminary trials by CAST IX suggest that hydrogel containing dinotefuran (0.05%) may remain active in the field for up to nine days when exposed to small amounts of precipitation. Light rain may be beneficial as it may slow desiccation and prolong bait attractiveness to YCA. In contrast, large amounts of rain may dilute the insecticide in the gel, rendering it ineffective. Applying hydrogel in the late afternoon or early evening during times when rain is expected to be minimal would be provide the best chance for the bait to be effective.

Birds on Johnston Atoll have not been observed feeding on hydrogel crystals, but concern exists that shorebirds, in particular, may consume hydrogel as they forage for terrestrial arthropods. Hydrogel toxicity towards birds is unknown, but ingestion might impede digestive processes. A buildup of hydrogel crystals in the environment would be expected with repeated application, and therefore multiple applications should be viewed with caution until more information about the natural decomposition of the crystals is available. Exposure to ultraviolet radiation, salt from sea spray, and physical abrasion from wind will likely fragment the crystals, and microbes such as bacteria and fungi are thought to contribute to the decomposition of polyacrylamide polymers (Chalker-Scott 2007). However, the time required before crystals are fully degraded, or at least no longer of perceptible size, is unknown.

Due to concern for the persistence of hydrogel crystals in the environment, textured vegetable protein (TVP) was tested as an alternative carrier for insecticide. TVP is a high-protein meat substitute made from defatted soy flour and is expected to degrade relatively rapidly in the environment. Analogous to hydrogel crystals, it is readily reconstituted by water and its solutes. TVP containing dinotefuran at 0.1% and 0.05% worked as well as hydrogel at reducing numbers of YCA on large plots, suggesting it may be a viable alternative, or supplement, to hydrogel. It is unclear if the vegetable protein adds to the attractiveness of TVP. Further study is needed to evaluate how long it remains attractive to, and effective against, YCA on Johnston.

Counts of ants within 0.5 m^2 quadrats were more effective at detecting YCA at low density than were bait tiles containing spam. In both small and large plots, bait tiles failed to identify YCA when quadrat counts consistently detected small numbers of ants. It is not clear whether YCA abundances were simply too low to effectively detect ants or if ants actively avoided the bait. Regardless, this result indicates that spam alone is unreliable for monitoring YCA at low abundance across the landscape. Hand-searching, whether using quadrats or other methods, is likely a more reliable method. This finding is important for developing strategies to evaluating the effectiveness of bait over large areas of the island.

Hydrogel containing dinotefuran (0.05%) was highly effective at reducing YCA abundances at all three application rates tested during the 19 May 2015 large plot trials, suggesting that the minimum effective concentration was not attained and that effective rates lower than 6 l/0.25 ha may yet be identified. Nevertheless, further testing is required before lowering the application rate because YCA numbers were not reduced during the third treatment on one large plot (43G) on which the low 6 l/0.25 ha rate followed two applications of thiamethoxam (0.01%). It is unclear why the reduced application rate failed during the earlier trial but may have been due to an overall aversion of YCA to any bait if thiamethoxam had been detected during the previous two treatments. Considering the contradictory result at the lowest application rate, it is most prudent to apply bait at a rate higher than lowest when treating at a larger scale.

Summary

Hydrogel containing insecticide dissolved in sucrose solution was highly effective at reducing YCA abundance in small and large experimental plots. Unexpectedly, none of the concentrations of thiamethoxam tested was found to be consistently effective at killing YCA in large field plots. In contrast, dinotefuran (0.05%) in hydrogel, was highly effective in all cases, warranting its usage on a large scale. TVP was tested less rigorously than hydrogel, but also appears to be an effective carrier of dinotefuran. Hydrogel bait applied on large plots at weekly intervals was increasingly effective over time, indicating a cumulative benefit with no aversion to the bait by YCA. This finding suggests that the sucrose matrices could be used at the landscape level in relatively rapid succession or in sequence with the cat food bait.

Suggestions for future work on Johnston Atoll

Results from CAST IX provide a promising framework for attempting to eradicate YCA on Johnston Atoll (Figure 13). Based on these results, an infestation-wide treatment strategy may take several forms, and could involve the use of hydrogel alone or in concert with the cat food bait. In addition, TVP may provide an alternative to hydrogel if the crystals are accumulating on the ground at a level that warrants concern. Regardless of the baits chosen, several aspects are critical for an eradication effort to succeed. These include:

- Determine accurately the boundaries of infestations. In particular, identify satellite populations outside the main infestation areas.
- Apply bait effectively over the treatment area. More specifically, apply relatively uniformly within ant habitat (i.e. areas other than pavement) and during periods of favorable conditions such as late afternoon and during periods of little or no rain. It is particularly important to apply bait around bases of trees and shrubs where nests are most likely to be located.
- Treat areas more than once because a single application is unlikely to succeed at killing all queens within nests.
- Detect low densities of YCA. This is critical to assessing the success of each application and in eradicating isolated populations. Additionally, target areas where ants are most likely to be found (e.g., the base of shrubs and trees; clumps of grass growing in pavement cracks) during monitoring (baits and hand-searching).

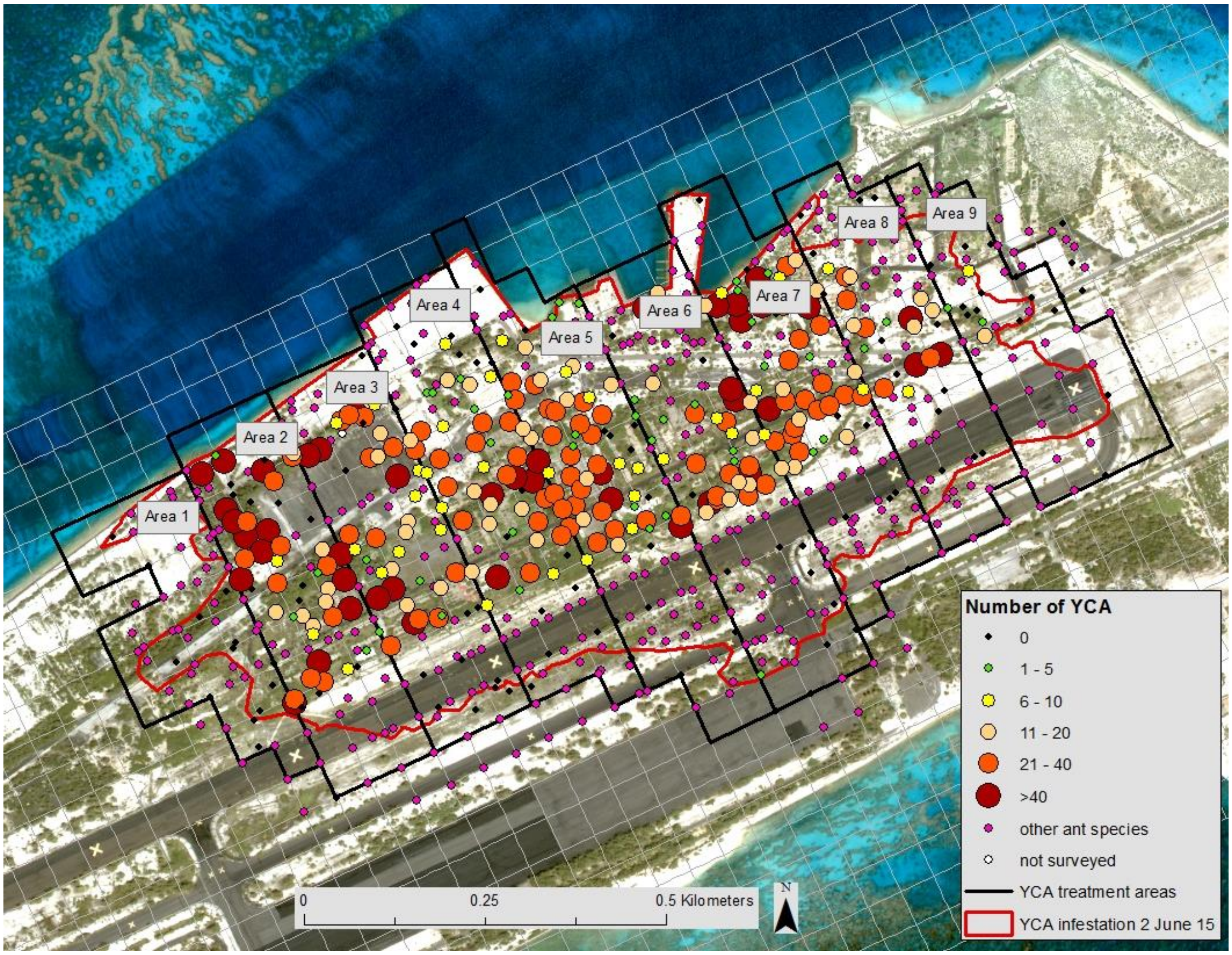


Figure 13. Abundance of YCA at monitoring stations within the infestation area during surveys conducted 25 May and 2 June 2015. Abundance data include results from the 50 x 50 m survey (baits at grid corners) and the treatment monitoring effort (baits located at points most likely to harbor ants). The monitoring lure (spam mixed with water) and baiting protocol was identical to that used in our experimental trials. The infestation area has been divided into nine potential treatment areas each about 8.5 ha in size (range: 6.75–10.25 ha).

Results from the small and large plot experiments are promising, but replication of the trials was limited, and therefore the initial phases of an infestation-wide treatment approach are more appropriately considered "experimental," with initial results influencing future strategy. In particular, it is unclear whether hydrogel is best used alone or as a supplement to the cat food bait. At this point, a dual approach would seem suitable, with part of the infestation treated with hydrogel alone and another part treated with the combination of cat food and hydrogel. Therefore, Phase 1 of the treatment strategy would consist of two large-scale applications, with one area receiving two treatments of hydrogel and the other receiving one treatment of cat food bait followed by one treatment of hydrogel. To effectively evaluate the treatments at the landscape level, the size of each of these treatment areas will need to be large. For each treatment area, the two applications of bait would likely be most effective if spaced about two weeks apart. Assessment following each application of bait would be critical for planning additional treatments.

The infestation may best be managed as a series of treatment areas, each of which can be treated with bait independently. For example, the infestation could be subdivided into nine areas (Figure 13), each approximately 8.5 ha in size and consisting of about 34 cells, each measuring 50 x 50 m. During Phase 1, areas 1 and 2 could be treated with dinotefuran in hydrogel alone and areas 3 and 4 could be treated by a combination of dinotefuran in cat food and hydrogel. Based on the results of Phase 1, subsequent treatments would then sweep across the infestation until YCA have been eradicated from all nine treatment areas.

Based on CAST IX experience, hydrogel bait can be applied at a rate of about 16–24 cells (50 x 50 m) per day and cat food can be applied at a rate of about 12 cells per day. At that rate, treatment of the entire infestation would take 12–19 days using hydrogel bait and 26 days using cat food bait. Based on the average treatment area of 33.9 cells, each treatment area could be completed in 1.4–2.1 days using hydrogel and 2.8 days using cat food. However, the total amount of area identified for treatment is greater than necessary as some cells include significant amounts of water and artificial surfaces, such as asphalt and concrete. For example, 26 of the cells are comprised almost entirely of the runway, of which only cracks would need to be treated, reducing the effort by overall area of coverage by about 8%. A possible way to partition the effort would be to consider the runway and those cells south of it as a discrete treatment area to be addressed independently. Doing so would allow the effort to initially focus on the part of infestation where YCA are currently known to exist.

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