The Management and Control of the Invasive Alien Crazy Ant (*Anoplolepis gracilipes*) on Christmas Island, Indian Ocean: The Aerial Baiting Campaign September 2002 – An Appraisal of Project Objectives and Key Outcomes.

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10 December 2002

In the Environmental Assessment that was prepared prior to the aerial baiting campaign, Green et al. (2002) listed three specific objectives of the aerial control program for crazy ants on Christmas Island. In this document, we evaluate the key outcomes of the aerial baiting campaign against these objectives, and summarise mopping-up operations after the aerial campaign. A full report on the aerial baiting campaign is almost complete, and will be available for circulation by the end of January 2003.

1. ***To trial the aerial dispersal of toxic ant bait (fipronil at 0.1 g kg^{-1}) for the control of Anoplolepis supercolonies on Christmas Island in August/September 2002;***

   (a) *to assess the efficacy of bait delivery by helicopter for controlling crazy ant supercolonies, by monitoring ant activity at replicate trial sites both before and after bait application;*

   (b) *to trial two bait application rates (4 & 6 kg ha^{-1}) at replicate sites, to identify the lowest effective rate for supercolony control;*

Completed. Eight treatment plots (15-53 ha) and two unbaited control plots (8-10 ha) were established in the south west of the island, and treated on 8 & 9 September 2002. There were four treatment combinations (high concentration bait/high application rate, high concentration/low application rate, low concentration/high application rate, and low concentration/low application rate), with n=2 replicate plots per treatment. Four parallel transects, each 150 m long and about 40 m apart, were established in all plots. Crazy ant activity was monitored at stations 15 m apart along the transects (11 per transect, 44 per plot at each census date). At each station, the number of ants running across the borders of a 10 cm x 10 cm white plastic card in 30 s was counted using a wrist watch and hand-counter. Counts were made on plots treated with high concentration bait three times (7, 5 and 1 day) before treatment and at eight times (2, 4, 6, 12, 19, 30, 37 and 44 days) post treatment. Counts were made on plots treated with low concentration bait at 7, 5 and 3 days before treatment, and at 2, 4, 8, 15, 22, 29, 36 and 43 days post treatment. Counts were made on the unbaited control plots on the same schedule as the high concentration plots throughout the monitoring period.

We estimated the mean application rate for each trial plot as the total (wet) mass of bait used to treat the plot (total wet mass of bait placed in the bucket suspended below the helicopter - wet mass of bait remaining at the end of baiting the plot), divided by the area of the plot. The rate at which the helicopter applied bait over the trial plots was close to the target rates of 6 & 4 kg ha^{-1}; mean application rates, on a wet mass basis, were 5.8 kg ha^{-1} and

4.4 kg ha^{-1}, respectively. These rates are significantly different (t-test, $t_{2\text{-tailed}}=9.760$, $p=0.000$).

The primary aim of the trial was to assess, within a matter of days, the lowest effective application rate of high concentration bait to achieve a large knock down effect on crazy ant worker activity. We did this in two stages; first, we confirmed through formal analyses that both application rates did in fact achieve a statistically significant knock-down effect. Separate repeated measures ANOVAs were performed comparing the time-course of ant activity on the controls against both the high and low application plots, for periods up to 8 days (3 counts) before and after treatment. The key test here is the time x treatment interaction; a significant interaction term implies a statistically significant impact of the bait on ant activity. However, these tests only confirmed the presence or absence of an impact *per se*, not its magnitude. Therefore, the second step was to compare the degree to which ant activity declined on high vs low application rate plots. Mean ant activity after treatment (average of three counts at 2, 4 and 8 days after treatment, where each count was the mean of two replicate plots) was compared to mean ant activity before treatment (average of three counts at 7, 5 and 3 days before treatment, where each count was the mean of two replicate plots).

Aerially-dispersed, high concentration ant bait had a significant negative impact on crazy ant activity within days of treatment, at both rates of application (Fig. 1a & 1b). Ant activity on the control plots was high during the week preceding treatment (mean counts of 60-80), and remained high during the week after treatment. Conversely, although overall ant activity on both the high and low application rate plots was lower in the week preceding treatment (mean counts 15-25), there was a significant decline in ant activity in both treatments during the week after baiting (time x treatment interaction for high application rate $F=8.58$, $p=0.002$; time x treatment interaction for low application rate $F=19.91$, $p<0.001$), such that activity was near zero in both treatments by 8 days after baiting.

The high and low application rates were equally effective at knocking down worker ant activity; compared to mean pre-baiting activity, post-baiting ant activity had declined by 91.2% and 94.4% on the high and low application rate plots, respectively, a little more than one week after baiting. Crazy ant activity was essentially nil in both treatments by 12 d after treatment.

(c) to assess the degree of bait penetration through the canopy to ground level at replicate trial sites;

Completed. A total of 30 catch bags, each with a collecting area of 1 m^2, were erected at 22-25 m intervals along the transects in each of the eight treatment plots. The bags consisted of a circular wire hoop supported 1.3 m above the ground on two wooden stakes. A large plastic bag was stapled onto the wire hoop, and sealed at the bottom with a rubber band. Tanglefoot™ was applied to base of each stake to exclude crazy ants. The bags were placed in their wire hoop frames about 2 - 3 hours before the helicopter treated each plot, and carefully emptied 3-4 hours after treatment. On the assumption that bait may have been caught in the canopy and then been dislodged later on, we emptied the catch bags a second time, either 3 (high concentration plots), or 4 days after treatment (low concentration plots). The samples were returned to the laboratory at PAN headquarters, where they were sorted under a stereomicroscope to isolate pellets of bait. The bait samples were dried at 60^{o}C for 5 days, and weighed. These samples were used to estimate the absolute mass of bait falling through the canopy to ground level.

The mean mass of bait falling into the catch bags over a three or four day period after treatment varied between 272 and 400 mg m^{-2} on the high application plots, and between 227 and 250 mg m^{-2} on the low application plots (Fig. 2). The vast majority of the bait fell straight through the canopy; pooled across plots, 192 of 240 catch bags intercepted 90% or more of their eventual 3 or 4 day totals by the first collection, just hours after treatment, while 164 of 240 catch bags intercepted 95% or more of their eventual totals within the same period. Within plots, the total mass of bait falling into the catch bags over the 3 or 4 day period varied widely; the coefficients of variation for all plots were 45% or greater.

These catch bag data are for oven-dry masses, but the mean rates of application reported above were for undried bait. In order to make them comparable, we estimated the mean moisture content of the bait used to treat each of the trial plots, so that the mean application rate for each plot could also be expressed on a dry mass basis. The mean moisture content of the bait varied from 6.6% on Plot A to 7.8% on Plot C (mean of all plots = 7.2%). Using these data, the dry mass application rates varied from 508 to 560 mg m^{-2} on the high application plots, and from 397 to 430 mg m^{-2} on the low application rate plots (Fig. 2).

For each plot, the proportion of dispersed bait that fell through the canopy to ground level was calculated as the mean dry mass of bait collected from the 30 catch bags after 3 or 4 days, divided by the average dry mass rate of application. The mean rate of bait penetration was 57.7% ± 7.0% (mean ± SD) across all plots. As these data suggest, most catch bags caught much less bait than was dispersed by helicopter above them, but between 2 and 6 bags on each plot (total 30 across all plots) intercepted more bait than their “target” masses, as defined by the average application for each plot.

(d) to trial the use of a lower-concentration bait (0.01 g kg^{-1}) for crazy ant control after 2002;

Completed. A secondary aim of the trial was to test, for future use, the efficacy of low concentration ant bait for controlling crazy ant supercolonies. We performed separate repeated measures ANOVAs comparing worker ant activity on the controls against both the high and low application plots, over the full time-course (44 d) of the experiment. As before, these analyses confirmed whether or not the bait had an impact *per se*, not its magnitude. Following these tests, we compared mean worker ant activity among the low concentration treatments at the very end of the monitoring period (4-6 weeks after treatment) to that in the most effective high concentration treatment, identified above.

Aerially-dispersed, low concentration ant bait had a significant negative impact on crazy ant activity, at both rates of application (Fig 1c & 1d; time x treatment interaction for high application rate $F=4.88$, $p=0.001$; time x treatment interaction for low application rate $F=28.04$, $p<0.001$). Ant activity in both treatments essentially fell to nil by 15 days after treatment, and remained at or near zero for the remainder of the monitoring period. The low concentration bait was slower-acting than the higher concentration bait. Mean ant activity typically fell by around 80% within 2 days of treatment on the high concentration plots, but only by around 50% on the low concentration plots in the same period of time. Eventually however, the lower concentration bait, even at the lowest rate of application, was just as effective at controlling crazy ants as the high concentration bait at the higher rate; mean ant activity had fallen to nil on all plots by three weeks post-treatment, and remained nil for the remainder of the monitoring period.

In a one-off survey six weeks after treatment (22 October 2002), we used sugar baits to more rigorously compare crazy ant activity on the high and low concentration treatments. We placed one 5 cm x 5 cm Wettex™ pad at each card counting station on the 150 m transects at each of the treatment plots (total of 44 pads per plot). These pads had first been soaked in a 30% v/v sucrose solution, and we recorded the duration (in minutes) that each pad was left on the ground. After an average of 30 minutes, we revisited each pad and counted the number of crazy ants feeding at it. To standardise these counts within and between plots, we expressed ant "density" as the number of ants recruited to the pad $minute^{-1}$. The average of 44 estimates per plot was used for analyses. Sugar baits were not placed on the control plots, because the primary aim of this exercise was to compare the treatments with each other, not with the controls.

Crazy ants occurred in *extremely* low densities on all experimental plots by the time of the sugar pad survey. A total (across all plots) of just 18 crazy ants recruited to the pads (44 pads/plot x 8 plots=352 pads in total), and on all the plots the mean number of crazy ants recruiting/min to the sugar pads was nil. From these data we conclude that although fipronil at 0.01 g kg^{-1} is slower acting that fipronil at 0.1 g kg^{-1}, eventually, both are equally effective at controlling crazy ant supercolonies on Christmas Island.

2. To eradicate all remaining Anoplolepis supercolonies from rainforest on Christmas Island by the end of October 2002;

(a) To disperse toxic ant bait (fipronil at 0.1 g kg^{-1}) by helicopter over all remaining crazy ant supercolonies on Christmas Island in August/September 2002;

Completed. Following the success of the trial, the Steering Committee approved (on 13 September 2002) the second stage of the aerial baiting campaign; the eradication of all known supercolonies on Christmas Island, using the higher concentration bait at a rate of 4 kg ha^{-1}. The committee also approved pilot discretion for the use of slightly higher application rates over areas of forest with relatively dense canopy. More than 2100 ha were treated by helicopter in the period 14-21 September. The only known supercolonies not treated by helicopter were several areas near freshwater streams and soaks (total untreated area 33.4 ha), and five Monash University research plots (total area 42.8 ha). The record of flight paths downloaded from the helicopter's differential GPS showed, in exquisite detail, that the pilot achieved blanket coverage in all target supercolonies, totalling more then 2500 ha. The only exception to this occurred in one small area at North-West Point, where there were too many flying sea birds for the helicopter to operate safely. After several attempts, the pilot was forced to abandon this area, leaving approximately 6 ha untreated.

(b) to monitor ant activity at 40 sites (island-wide) in ant-infested forest, two weeks before and two weeks after the helicopter campaign;

Completed. To assess the island-wide impact of the aerial baiting campaign on crazy ant supercolonies, we conducted pre- and post-baiting surveys of ant activity at 44 monitoring sites distributed among most supercolonies across the island. At each site, we completed standard 30 s card counts of ant activity at 11 stations spaced at 15 m intervals along a 150 m transect, identical to the methodology for assessing ant activity in the Trial plots. Ant activity at each site was assessed twice, once 13-36 days before (mean 26 d), and once 24-27 days (mean 28 d) after the sites were treated by the helicopter. The mean of eleven card counts at each site was used for analyses.

The aerial baiting campaign had a significant impact on crazy ant activity in supercolonies across the island. Mean ant activity across the 44 monitoring transects fell from 21.6 ± 18.7 (mean ± SD) prior to treatment, to just 0.13 ± 0.50 several weeks after treatment. This 166-fold decline was highly significant (paired t-test, $t=7.643$, $p=0.000$). The mean decline in ant activity on each 150 m transect was 99.4%, with 40 of 44 transects showing declines of 97% or greater. Ant activity declined to zero on 34 of 44 transects, and the lowest decline of any transect was still 92.7%. The impact of the aerial baiting campaign was density-independent; the degree of decline in ant activity was not dependent on the magnitude of pre-treatment ant activity ($r^2=0.001$, $p=0.824$, $n=44$). From these data we conclude that crazy ant supercolonies have been eradicated from all areas treated by helicopter in September 2002.

3. To achieve all the above objectives with the least possible impact on non-target species.

Completed. Data and observations indicate that very few non-target impacts occurred as a result of the aerial baiting campaign;

1. Accuracy of aerial treatment around the boundaries of supercolonies. Accuracy around the boundaries of crazy ant supercolonies was of paramount importance in reducing non-target impacts on susceptible fauna in areas adjacent to supercolonies. The record of the flight paths downloaded from the helicopter's differential GPS allowed us to assess, in minute detail, the accuracy of the pilot in treating supercolonies across the island. The pilot did a superbly accurate job – he never baited outside boundaries which lay parallel to his direction of flight, and only on several occasions did he overshoot boundaries at the end of baiting runs. However, the area of intact forest treated with ant bait as a result of these overshoots totalled less than 1 ha, in more than 2500 ha baited.

2. Sea-birds. Observations by observers stationed in the helicopter and on the ground indicated that Abbott's boobies were not disturbed by the helicopter flying above them. None of the birds under observation took flight or abandoned nests, and few showed signs of having noticed the aircraft at all. Red footed boobies and great frigate birds occasionally took flight as a result of helicopter operations. The pilot reported only a single incident of bird-strike, where a great frigate bird grazed the bottom of his aircraft over North-West Point.

3. Canopy arthropods. A research team from the Rainforest CRC (based at James Cook University in Cairns) was engaged to assess the impact of the aerial baiting campaign on canopy arthropods and vertebrates. Their final report is still pending, but preliminary analyses (N. Stork, unpublished data) indicate no detectable impact on canopy arthropods, and no immediate negative impacts on the endemic Christmas Island Whit-eye, the Christmas Island Thrush, or the endemic Christmas Island gecko.

4. Litter Invertebrates. A research project that assessed non-target impacts of the ground-based control program on Christmas Island (Marr 2002) found no evidence of non-target impacts on litter invertebrates, which she attributed mostly to bait monopolisation by crazy ants. Given this, it is highly unlikely that the aerial baiting campaign caused any substantive non-target impacts on litter invertebrates; both the aerial baiting campaign and ground-based

program used the same bait formulation with fipronil at 0.1 g kg^{-1}, and overall, the aerial campaign used a much lower rate of application (4.0 kg ha^{-1} in the aerial campaign) than the ground based program (6.0 kg ha^{-1}).

5. Red crabs, *Gecarcoidea natalis*; Fipronil is toxic to red crabs, but the risk posed to the red crab population by the aerial baiting campaign was considered minimal because

- within supercolonies, crazy ants annihilate the resident population of red crabs
- red crabs seem not to be attracted to Presto ant bait, and
- the aerial baiting operation was conducted under very dry conditions, which virtually confined red crabs to the interior of their burrows away from contact with the bait.

After the aerial baiting campaign, field crews reported seeing fewer than 10 dead (baited) red crabs during all of their searches of the Boundary plots for the robber crab mortality survey (see below).

6. Robber Crabs, *Birgus latro*; Fipronil is toxic to robber crabs, and they are attracted to Presto ant bait even in very dry conditions. To minimise this non-target impact, the helicopter was used to drop food lures at a total of 253 sites around the perimeters of target supercolonies. These lures were designed specifically to keep robber crabs living in uninvaded forest near supercolonies busy long enough for crazy ants to remove most of the toxic bait, reducing the risk to the crabs. These sites were spaced at intervals of 150 - 250 m, and lay 50 - 200 m outside supercolony boundaries. Approximately 15 kg of a chook food/shrimp paste mix was dropped by helicopter at each site one or two days before adjacent supercolonies were treated with ant bait.

We further reduced the potential for robber crab mortality by felling *Arenga listeri* palms at strategic locations around the perimeters of the supercolonies. It is well known that robber crabs find the fruit and pith of these palms irresistibly attractive, and cut palms were likely to be many times more effective at keeping robber crabs away from target supercolonies than chook food alone. We used chain saws to cut down a total of 45 palms several weeks prior to the commencement of the aerial baiting program. The palms were spaced several hundred meters apart and up to 300 m away from baited boundaries.

We conducted a survey in October 2002 to determine how successful our efforts had been to reduce the impact of the aerial baiting campaign on the *Birgus* population. Thirty sites, each 1.4 ha, were thoroughly searched by teams of people on foot between 26 September and 10 October 2002. These sites were arranged in triplets, and within each triplet, one site was centred on a felled *Arenga* palm several hundred meters from the baited edge of a supercolony, another site straddled the nearest baited boundary, and a third site was placed between these two. This design allowed us to estimate an approximate mortality rate for *Birgus* within several hundred metres of baited supercolonies.

A total of 831 robber crabs were found across all plots, including 42 dead animals, for an average mortality rate of 5% in the vicinity of supercolony boundaries. All of the dead animals were found in the Boundary plots, but mortality was patchy; within site triplets, mortality ranged from 0 to 13%. It was not possible for us to conduct similar surveys in areas that had not been prepared with chook food lures and *Arenga* palms, but surveys conducted in September 2000 as part of the ground-based control effort suggest that average robber mortality in the absence of such measures is around 15%.

Follow up Control of Supercolonies after the Aerial Baiting Campaign

As detailed above, several infested areas were deliberately not treated by the helicopter; areas near freshwater streams and soaks (total untreated area 33.4 ha), and five Monash University research plots (total area 42.8 ha). The former were not treated in the aerial campaign because of concerns over the ability of the helicopter to bait accurately around streams and soaks. However, teams on foot can deliver bait in a highly accurate manner to within metres of wet areas. In the weeks following the aerial campaign, the former exclusion zones at the Dales, Hosnie's Spring and Harrison's Spring were all baited on foot. In addition, 3 of the five Monash University research plots were also baited. The remaining two (total c. 15 ha) have been targeted for eradication as soon as weather and resources permit.

During October and November 2002, crazy ant supercolonies were detected at three other locations, unknown at the time of the aerial baiting campaign. These are at North-East Point on the shore terrace, Jack's Hill on the plateau, and on the western slopes of Middle Point. The Middle Point infestation has been partially baited, and all three new supercolonies are targeted for eradication as soon as weather and resources permit. The combined area of these infestations is unknown, but they are suspected to be relatively small.

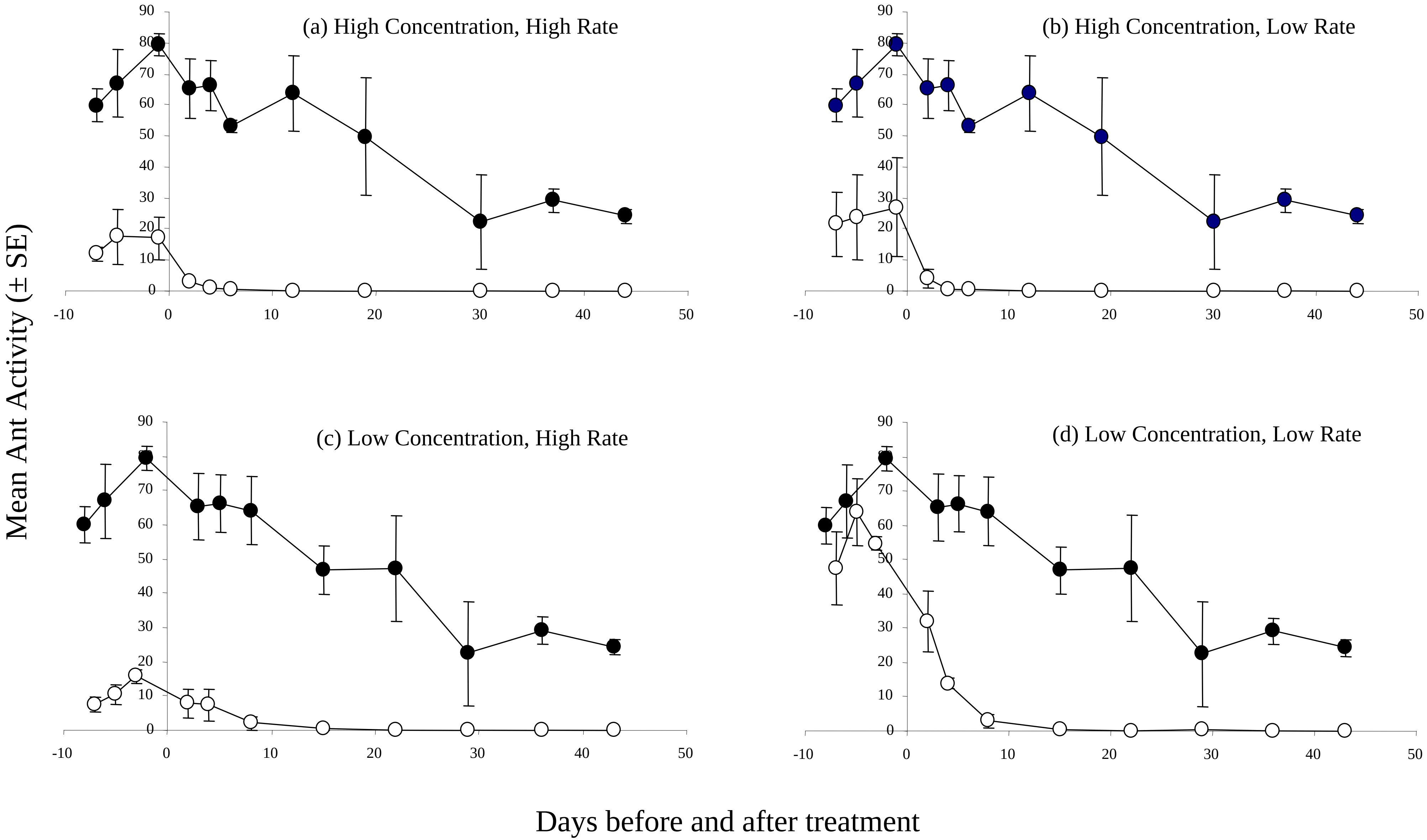

Fig. 1. Mean ant activity on (a) the high concentration, high application plots, (b) the high concentration, low application rate plots, (c) the low concentration, high application rate plots, and (d) the low concentration, low application plots, before and after treatment (day "0"). Filled circles are control plots (n=2), open circles are treatment plots (n=2). There were only two control plots, but to make comparisons easier, the data have been plotted for each combination of bait concentration and application rate.

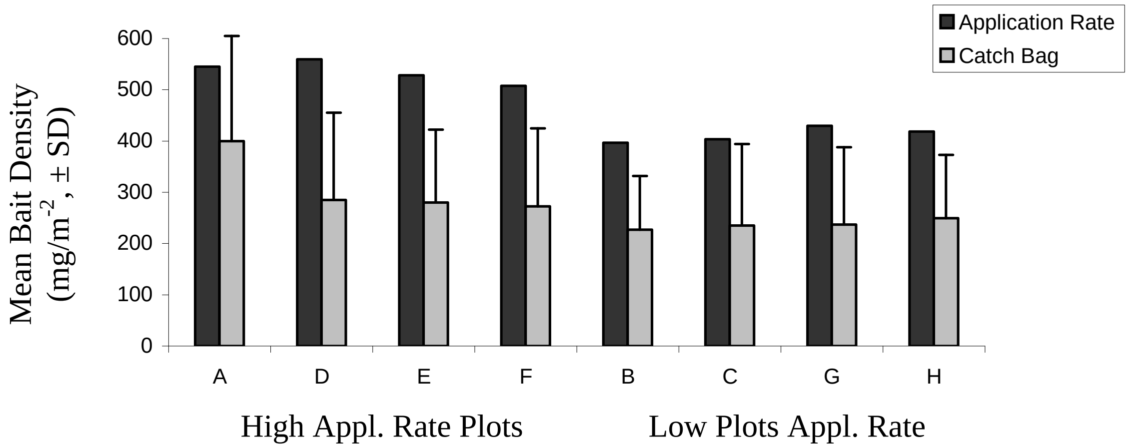

Fig. 2. The mean dry mass of bait intercepted by the catch bags over a 3 or 4 day period after treatment during the aerial baiting trial (pale bars), and the mean (dry mass) rate of bait application over the plots (dark bars).