Heli-baiting using low concentration fipronil to control invasive yellow crazy ant supercolonies on Christmas Island, Indian Ocean

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Abstract Yellow crazy ants (*Anoplolepis gracilipes*) invaded Christmas Island sometime before 1935. By 2001, the species had formed destructive supercolonies over 2500 ha, or almost 30% of the island's rainforest. A heli-baiting operation in 2002 used high concentration fipronil (at 0.1 g/kg at 4 kg/ha) to eradicate all targeted supercolonies. However, supercolonies began to steadily redevelop across the island. We conducted surveys over the entire island from May to September 2009 and located 74 separate supercolonies that covered 833 ha. The boundary of each supercolony was mapped precisely by ground truthing. Two thirds of this area was too inaccessible and dangerous to be baited using standard hand-baiting techniques. Thus, in September 2009 we heli-baited 785 ha of supercolonies (with the remaining 48 ha intentionally not baited), using 3294 kg of ant bait, but this time using one tenth of the previous concentration of fipronil (0.01 g/kg at 4 kg/ha). All targeted supercolonies were again controlled, with ant activity reduced by 98.4% four weeks after baiting, and remained reduced by 99.4% 20 weeks after baiting. Direct non-target impacts of the baiting were minimal.

Keywords: Bait efficacy; surveys; non-target effects; land crabs

INTRODUCTION

Yellow crazy ants (*Anoplolepis gracilipes*) are one of the world's worst invasive species (Lowe *et al.* 2000) and are now widely distributed throughout the tropics (Wetterer 2005). These ants were accidentally introduced to Christmas Island some time prior to 1934 (Donisthorpe 1935). Ant numbers remained extremely low and had no obvious effects on the island's biota for decades. However, like many other invasive species of ants (Suarez *et al*. 2001; Holway *et al*. 2002; Tsutsui and Suarez 2003), crazy ants can form unicolonial (multi-queened) supercolonies where extremely high numbers of ants forage on the ground and in the canopy of rainforest trees (e.g., Haines and Haines 1978; Feare 1999). On Christmas Island, yellow crazy ants appear to benefit from a mutualistic relationship with introduced sap-sucking scale insects (Coccidae and Kerriidae) that secrete abundant, energy-rich honeydew (Abbott and Green 2007). As a consequence of this mutualism, the density of foraging yellow crazy ants within supercolonies typically exceeds 2000 ants per m^2 (or 20 million ants per ha) on the forest floor alone with 10.5 nests per m^2, which is the highest recorded density of foraging ants (Abbott 2005).

The first supercolony on Christmas Island was discovered in 1989 near the island's urban area, "the Settlement", where about 2 ha of forest were infested with crazy ants. No increases in the abundance of supercolonies were reported until 1996 (O'Dowd *et al.* 1999), following which untreated supercolonies expanded around their entire perimeter at rates of ~0.5 m per day (Abbott 2006). By December 1998, the total known infestation approached 200 ha, comprising 2-3% of the rainforest on Christmas Island (O'Dowd *et al.* 1999). Within four years, crazy ant supercolonies expanded to cover approximately 2500 ha, or more than 28% of the remaining forest. At supercolony densities, yellow crazy ants cause a rapid catastrophic shift in the rainforest ecosystems of Christmas Island, particularly through their impact on the red land crab (*Gecarcoidea natalis*) (O'Dowd *et al.* 1999, 2003; O'Dowd and Green 2009; Smith *et al.* subm.; see also Davis *et al.* 2008, 2010). Controlling infestations of yellow crazy ants on Christmas Island is of utmost importance for Christmas Island biota (Commonwealth of Australia 2006a, 2006b). This evolving crisis prompted an emergency response from the Australian Government (Green *et al.* 2004; Green and O'Dowd 2009). In September 2002, fishmeal baits with an active constituent of fipronil at 0.1g/kg were spread by helicopter (heli-baiting) at 4 kg/ha over 2509 ha of supercolonies. The campaign reduced ant abundance by an average of 99.4% within four weeks at all treated supercolonies (Green *et al.* 2004; Green and O'Dowd 2009).

Supercolonies again began to develop steadily across the island despite Christmas Island National Park (CINP) field teams' hand baiting 210 ha of supercolonies per annum with fipronil. The hand baiting did not keep pace with the rate of supercolony formation, particularly on the many inaccessible cliffs. By September 2009, over 800 ha of supercolonies again existed across Christmas Island (CINP unpubl. data).

Previous efforts to control or eliminate crazy ant supercolonies relied upon a relatively high concentration of fipronil. Here we document the efficacy of a 2009 heli-baiting campaign, which is the first crazy ant control programme to use low concentration fipronil (0.01 g/kg at 4 kg/ha) over a broad area.

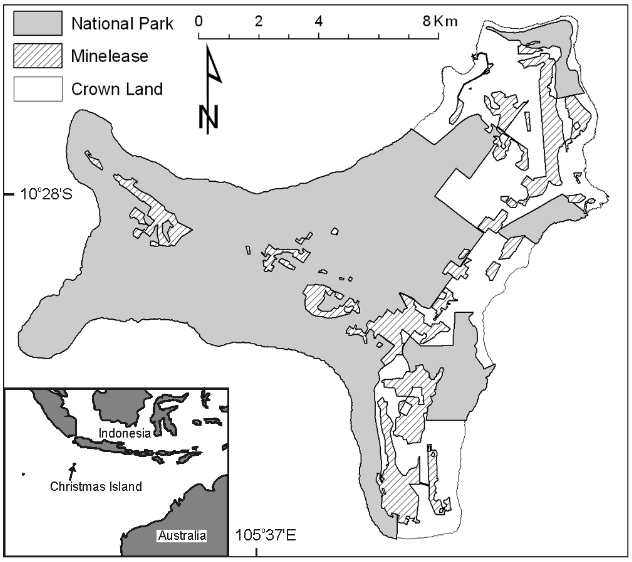

Fig. 1 Location and land tenure on Christmas Island.

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METHODS

Location

Christmas Island (10°25'S and 105°40'E) is an isolated oceanic limestone island of 135 km² in the north-eastern Indian Ocean 360 km south of Java and 2800 km west of Darwin. About 74% of the island is covered with natural vegetation comprised mostly of structurally simple, broad-leaved rainforest; 63% of the island comprises Christmas Island National Park (Fig. 1). The highest point is 361 m above sea level (Commonwealth of Australia 2006b). Christmas Island has a wet season from December to April, although rain may fall in any month of the year. Mean annual rainfall is 2068 mm, mean maximum temperature is 27.3° and the mean minimum temperature is 22.8° (Australian Bureau of Meteorology; Claussen 2005).

Field methods

Commencing in 2001, Christmas Island National Park conducted biennial surveys for yellow crazy ants, red crabs and other key biota at 877-1024 survey points (Fig. 2) spaced ~365.7 m apart across the entire island (Smith *et al.* subm.). Surveys were conducted during the dry season between May and September. At each survey point, teams of two field staff used two methods (one objective, one subjective) to assess whether that survey point fell within a supercolony. For a rapid, objective assessment of ant abundance, a 50 m transect was placed along the same bearing each year. These bearings were originally chosen randomly, although some were varied if extreme terrain made the site inaccessible. Each transect consisted of eleven sampling points located at 5 m intervals. At each sampling point, leaf litter was cleared with a swipe of the boot, and a laminated 20 x 20 cm card with lines dividing the card into four 10 x 10 cm quarters was placed on the cleared ground. One 10 x 10 cm quarter was selected at random. Observers then waited for 15 seconds before counting the number of ants that crossed the selected quarter over the ensuing 30 second period (cf. Abbott 2004; Green *et al.* 2004). Counting stopped if numbers exceeded 100 ants per 30 seconds. Counts were summed across the 11 card counts on each transect. Ant counts exceeding 37 ants per transect were identified as potential supercolonies because at these densities the ants tend to eliminate red crabs (CINP unpubl. data).

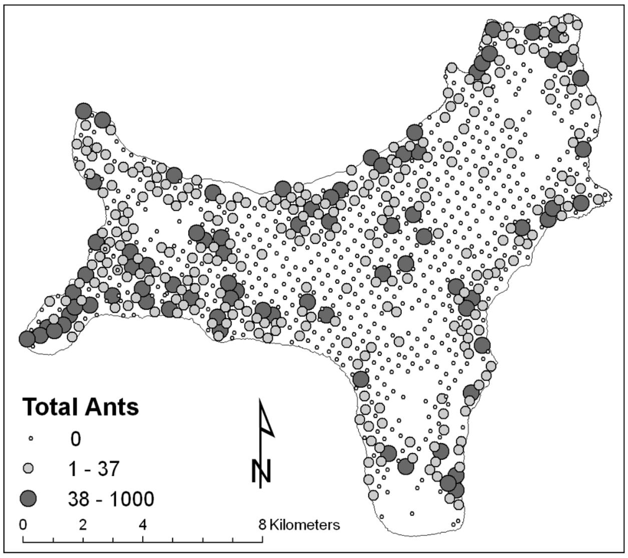

Fig. 2 Yellow crazy ant activity at 902 waypoints from the 2009 island-wide survey of Christmas Island. Black dots indicate waypoints with no ants recorded on ant activity cards; grey-centred circles indicate crazy ants were present but not in supercolony densities (1-37 ants on activity cards); large dark dots indicate that crazy ants were present at potential supercolony densities (>37 ants on activity cards).

At each survey point, and in transit between survey points, field teams also made subjective assessments of whether the area appeared to be a supercolony by looking for characteristic signs of crazy ant infestation including: 1) high crazy ant abundance on the ground and as 'trunk traffic' on trees; 2) large numbers of ant nests, typically at the base of trees and in rotten logs; 3) ant-infested red crab burrows; 4) dead red crabs (or other dead land crabs); 5) relatively large amounts of leaf litter; 6) relatively high numbers of scale insects; 7) excessive sooty mould; 8) giant African land snails; 9) relatively high numbers of seedlings; and 10) a relatively low diversity of 'other invertebrates', particularly 'other ants'.

The locations of any potential supercolonies discovered in transit between any of the survey points were recorded on hand-held GPS units (Garmin GPSmap 60CSx).

Following the objective and subjective assessments, each waypoint was then categorised as: 1) ants absent; 2) ants in low density; or 3) ants in a potential supercolony (on the basis of *either* of the objective or subjective assessment methods). These data were then used to generate a distribution map of potential supercolonies (Fig. 2) via ArcGIS 9.3.2.

Each potential supercolony was then revisited by field teams who mapped the precise location of its boundaries as follows. Three people walked 5-20 m apart along the length of the boundary with one person 'inside' the supercolony boundary continually searching for and confirming the *presence* of high densities of ants (and the supercolony characteristics listed above); one person 'outside' the supercolony boundary continually searched for and confirmed the *absence* of high numbers of ants; while the third person held the middle ground between the other two searchers. Through constant communication, the two outer people kept the middle person accurately positioned on the supercolony boundary. The person in the middle marked the boundary coordinates every 10-30 m using GPS and a hip-chain stringline to define a biodegradable cotton marker boundary to the supercolony. Most boundaries are easily identifiable by field crews on the ground. Occasionally, however, there was a wide 'transition zone' (cf. Abbott 2006) between heavily infested forest with high densities of ants and no live red crabs before reaching intact forest with very few or no crazy ants and many live red crabs. Although delineating the boundary required subjective assessment (particularly colonies with wide transition zones), the effectiveness and accuracy of this technique was regularly demonstrated as field crews – regardless of the size and complexity of the supercolony – always returned to within metres of the starting point.

Because of the fluid nature of supercolony boundaries, their perimeter needed to be delineated as soon as possible before the actual heli-baiting. Thus, boundary marking began on 4 August 2009 and continued until 16 September 2009, with the last of the supercolony boundaries being delineated while heli-baiting was under way elsewhere (see below). This methodology produced up-to-date detailed maps of every crazy ant supercolony on the island, with very finely resolved boundaries (Fig. 3).

Heli-baiting

AntOff ant bait, with the active ingredient fipronil at 0.01 g/kg, was supplied by Animal Control Technologies

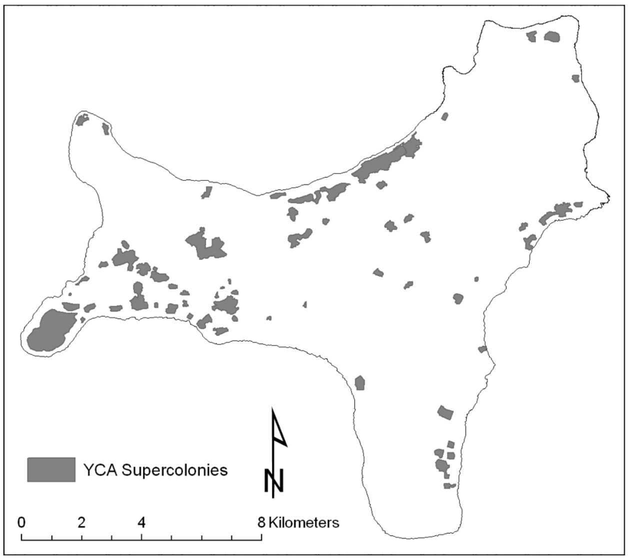

Fig. 3 Distribution of yellow crazy ant supercolonies following boundary ground-truthing by field teams prior to heli-baiting in September 2009.

(Australia) Pty Ltd in the form of small pellets, roughly 1.5 x 1.5 mm in cross section, and between 2 and 6 mm long. The 7000 kg of bait was packaged in 12.5 kg plastic-lined cardboard boxes and transported to Christmas Island by ship.

Heli-baiting was planned for September 2009, the end of the dry season. This month was chosen because: 1) bait delivery and bait uptake by the crazy ants would be impeded during wet weather; 2) land crab activity is minimal at the end of the dry season as red crabs tend to remain in their burrows, thereby reducing the potential for non-target contact with the bait; and 3) the rainforest canopy is at its most open enabling more bait to fall to the forest floor (cf. Green *et al*. 2004).

AntOff baits were dispersed over supercolonies from a Bell 47 Soloy helicopter operated by McDermott Aviation Pty Ltd. The bait delivery mechanism used was developed by McDermott Aviation for the 2002 heli-baiting operation on Christmas Island and described by Green *et al*. (2004) and Green and O'Dowd (2009). Essentially, bait was dispersed from an inverted conical bucket suspended below the helicopter. Bait flowed through a 25 mm diameter aperture in a base plate at the bottom of the bait bucket and onto a rotating spreader powered by a petrol-driven, four-stroke engine attached to the framework of the bucket. Pilots entering the air space above a supercolony boundary electronically opened a sliding gate beneath the aperture in the bucket, thereby enabling the bait to flow onto the spreader. This resulted in an even spread of baits for 12 m either side of the helicopter at a rate of roughly 4 kg per hectare when the pilot flew at 100 km per hour.

Supercolony boundaries were defined for the helicopter pilots on ArcMap layers. The pilot used a Trimble differential GPS unit with sub-metre accuracy to ensure that baits were spread to the edge of supercolonies and that flight paths were straight and the correct distance apart, which gave continuous and even spread of bait over the entire target supercolony.

Five supercolonies or subsections of supercolonies, each about 5 ha, were deliberately left unbaited for an ongoing research project into biocontrol of scale insects (a joint collaboration between the Director of National Parks, Christmas Island National Park, Monash University and La Trobe University). Three small supercolonies (9 ha total) on a steep slope near the township were baited by hand because the local community raised concerns about human safety. One supercolony and one subsection of a supercolony were not treated because they were intentionally set aside for an ongoing alternative baiting research project (2 ha). Because fipronil can have strong negative effects on freshwater fauna (e.g., Maul *et al*. 2008), we did not bait two supercolonies (12 ha) that were within 200 m of Ramsar Wetlands of International Importance (Hosnie's Spring and The Dales). In total, 48 ha were not treated during this heli-baiting campaign.

One non-target species susceptible to fipronil is the robber crab (*Birgus latro*), which is attracted to AntOff baits (CINP unpubl. data). In order to minimise robber crab mortality, we created food lures designed to entice them away from baited sites. In the weeks prior to heli-baiting, 4000 kg of chicken feed pellets was mixed with 320 kg of shrimp powder ('Belacan') in concrete mixers and placed into 12 kg bags. These bags were stored for as long as possible, which allowed the shrimp powder to infuse with the chicken food pellets. One or two days before heli-baiting, the helicopter was used to drop lure stations (3-4 kg of chicken feed / shrimp powder mixture) at intervals 50 m from the mapped supercolony boundaries. Lure stations were delivered from a different bucket slung beneath the helicopter to ensure that the chicken pellets were not contaminated with any residual fipronil. This method effectively lured most robber crabs out of areas to be baited (CINP unpubl. data). In total, 1105 robber crab lure stations were deposited from the helicopter.

Christmas Island National Park has engaged CESAR Consultants Pty Ltd., as independent consultants to quantify direct and indirect (bioaccumulation) impacts of baiting on non-target species. These data are still being collected.

Monitoring bait efficacy

The effects of aerial baiting on ant density were assessed at nine supercolonies (Table 1), which provided a range of densities and locations across the island. In addition, the four most accessible of the five untreated biocontrol research project plots were used as control sites to monitor the density of ants without chemical treatment (Table 1).

Estimates of ant densities in trial supercolonies were obtained using standard Christmas Island National Park methods employed since 2001: 3 x 50 m straight line transects were established within the boundary of each

Table 1 Mean pre-treatment ant densities and areas of monitored supercolonies.

Supercolony ID	Initial Ant Density	Area (ha)	Treatment
917	24	25.7	Baited
372	67	100.7	Baited
135	69	12.5	Baited
368	88	5.3	Baited
538	102	4.4	Baited
467	144	8.1	Baited
252	158	30.5	Baited
148	174	29.0	Baited
184	528	63.1	Baited
403	182	5.4	Control
582	200	4.8	Control
318	238	5.2	Control
206	414	4.8	Control

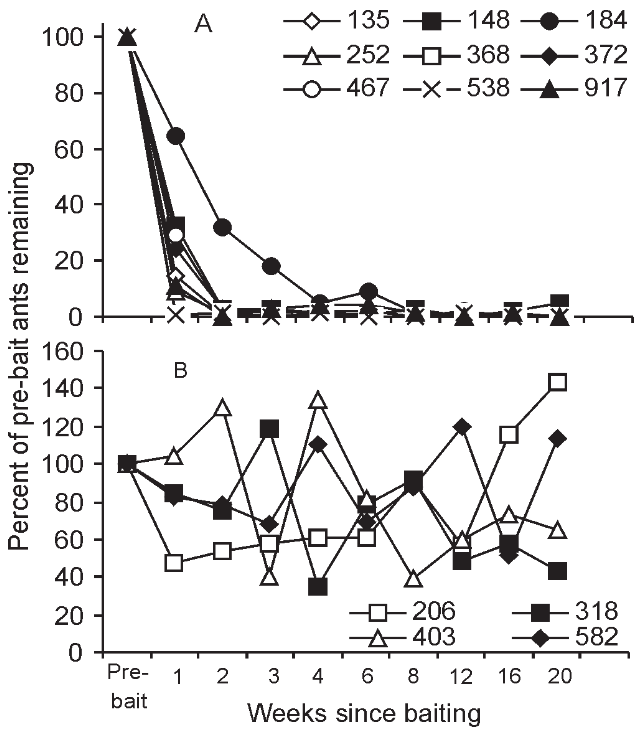

Fig. 4 Ant activity at **(A)** nine baited and **(B)** four unbaited (control) supercolonies expressed as a percentage of the of mean ant activity for three weeks prior to baiting for each supercolony. Numbers in the legend indicate supercolony identity.

supercolony; each transect was located at least 50 m from a boundary, and at least 50 m from a neighbouring transect; and each transect consisted of eleven survey points at 5 m intervals marked with flagging tape. At each survey point, ant cards were used to estimate ant activity per 30 seconds using identical methods described above for the island-wide survey of ants. Counts were summed across the 11 card counts on each transect. Ant activity was defined as the mean of ant counts from the three transects within each supercolony. Ant counts were conducted weekly for three weeks prior to baiting (to obtain a pre-baiting mean density), and then 1, 2, 3, 4, 6, 8, 12, 16 and 20 weeks after baiting.

RESULTS

Surveys for crazy ants over Christmas Island revealed that 542 of the 902 (60.1%) waypoints had ant infestations with potential to become supercolonies (Fig. 2). Ground truthing of all potential supercolonies and those discovered in transit revealed 74 discrete supercolonies covering 833 ha (Fig. 3), with supercolony area between 0.4 and 141.1 ha (mean 11.5 ha). Forty-two supercolonies covering 511.9 ha (or 65.2% of total heli-baited area) were in areas too rugged to bait by hand.

Baits were spread from 4-19 September 2009 covering all 784.8 ha of targeted supercolonies with 3294 kg of bait at a mean application rate of 4.2 kg per ha. GPS downloads revealed remarkably few inaccuracies during the aerial baiting campaign, with no baits being spread outside the targeted areas.

Ant densities declined by a mean of 79.3% (±20.1 SD) one week after baiting and 98.4% (±1.9 SD) four weeks after baiting (Fig. 4a). This reduction was sustained, with ant numbers reduced by 99.4% (±1.6 SD) some 20 weeks after baiting (Fig. 4a), when 288 of 297 (97.0%) ant sampling points still had zero ants per 30 seconds on ant count cards. Ant activity in control plots remained high, although it varied over the monitoring period (Fig. 4b).

Within baited supercolonies, the percentage decline in ant activity one week after treatment was negatively correlated with log pre-baiting ant activity (linear regression $F_{1,9}$=6.4; P=0.04); low density crazy ant supercolonies declined more rapidly than high density supercolonies. For example supercolony 184 initially had an average of 528 ants per transect and declined more slowly than supercolony 368, which initially had 86 ants per transect (Fig. 4).

DISCUSSION

The yellow crazy ant heli-baiting campaign on Christmas Island in 2009 was a complete success. The entire island was surveyed for ants, all supercolonies were delineated, all targeted supercolonies were heli-baited on time, and, importantly, all monitored supercolonies showed decreases in ant activity to well below supercolony level. Within four weeks of baiting, virtually no crazy ants were recorded on ant activity cards within baited supercolonies, and this pattern has continued for the first 20 weeks after baiting.

This is the first attempt to control yellow crazy ants on a broad scale using fipronil at 0.01 g/kg at 4 kg/ha. For example, in Arnhem Land, northern Australia, yellow crazy ant supercolonies are treated with 0.01 g/kg at 10 kg/ha (B. Hoffman pers. comm.). Between 2000 and 2009, Christmas Island National Park used fipronil at 0.1 g/kg at between 4 kg /ha (e.g., Green *et al*. 2004; Green and O'Dowd 2009) and 6 kg / ha (CINP unpubl. data). These higher doses were understandable given the urgency and novelty of the yellow crazy ant situation in 2001, where almost 30% of the island had become heavily infested with crazy ant supercolonies (Green and O'Dowd 2009), and failure to control the supercolonies would have been disastrous for the Christmas Island biota. In the 2002 heli-baiting campaign, Christmas Island National Park achieved a 99.4% knockdown of yellow crazy ants in all monitored supercolonies (Green and O'Dowd 2009). We achieved an identical knockdown (99.4%) using a ten-fold lower concentration of active ingredient. In total, 31 g of fipronil was used to eradicate 785 ha of supercolonies.

It may be possible to further reduce the concentration of fipronil used to control supercolonies, particularly those with less dense ant populations. For example, supercolony 184 had the highest density of ants recorded on the island. Despite the lower concentration of fipronil used in this programme, this supercolony was eradicated within four weeks.

Christmas Island National Park has been conducting chemical baiting trials since 2000 to determine the most effective method of controlling yellow crazy ants (CINP unpubl. data). Despite trialling hydramethylnon, pyriproxyfen and indoxacarb, fipronil has proven to be the only effective option for controlling yellow crazy ants on the island. Surprisingly, hydramethylnon effectively eliminated yellow crazy ant supercolonies in Arnhem Land (B. Hoffmann pers. comm.).

Fipronil is a phenylpyrazole broad spectrum insecticide effective at low field application rates against a wide range of arthropods (including crustaceans), even those often resistant to other insecticides, such as pyrethroids, organophosphates and carbamates (Narahashi *et al.* 2007).

However, it is unlikely that the heli-baiting campaign on Christmas Island heavily affected non-target species for several reasons. First, Christmas Island National Park only treats high density ant infestations (i.e., supercolonies). In these areas, non-target impacts are minimal since most native invertebrates have already been killed by the crazy ants. Furthermore, crazy ant activity is so high in such areas they remove bait at rates of 7% per minute (Marr 2003), which limits bait exposure to surviving native species. Christmas Island National Park can not apply fipronil to areas containing crazy ants at low densities because the non-target impacts would be catastrophic.

One native invertebrate that can enter baited supercolonies is the large (up to 6 kg), nomadic robber crab. This species usually survives for some time as it passes through a crazy ant supercolony but is also highly susceptible to fipronil poisoning. We used lure stations around selected supercolonies to attract robber crabs and found more than 100 individuals at one lure station within 24 hours of placement. There was low mortality of crabs around baited supercolonies even where crabs were known to be adundant nearby (CINP unpubl. data). Further, no red crabs were found dead within or around baited supercolonies. Either the red crabs were not sufficiently attracted to the AntOff bait to emerge from their burrows during the heli-baiting campaign or the yellow crazy ants monopolised baits before red crabs from outside the supercolony could locate them.

Data collected during the 2002 heli-baiting campaign indicated that most of the aerially-delivered ant bait successfully passed through to the forest floor. If bait remained within the forest canopy, it was most likely to be consumed by crazy ants (Green and O'Dowd 2009). There was no evidence of an impact of fiprinol on native canopy arthropods, arboreal geckoes or land birds (Stork *et al.* 2003), nor was there any evidence of impacts on native leaf litter invertebrates (Marr 2003). There was no residual fipronil detected in the soil one week, one year or two years after aerial baiting in 2002 (Marr 2003). Given that we used fipronil at a lower concentration, we expected even fewer non-target impacts from the 2009 heli-baiting campaign.

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