Technical Report HCSU-071

# ARIAL-BROADCAST APPLICATION OF DIPHACINONE BAIT FOR RODENT CONTROL IN HAWAI`I: EFFICACY AND NON-TARGET SPECIES RISK ASSESSMENT

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November 2015

USGS science for a changing world

University of Hawai'i Hilo

This product was prepared under Cooperative Agreement CA03WRAG0036 for the Pacific Island Ecosystems Research Center of the U.S. Geological Survey.

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## ABSTRACT

Introduced rats (*Rattus rattus, R. exulans,* and *R. norvegicus*) have been implicated in the decline or extinction of numerous species of plants and animals in Hawai'i. This study investigated the efficacy of aerial-broadcast application of Ramik® Green baits containing 50 ppm (0.005%) diphacinone in reducing rat and mouse populations and the risk to non-target species. The study was undertaken in paired 45.56-ha treatment and non-treatment plots in Hawai'i Volcanoes National Park. All 21 radio-collared rats in the treatment plot died within nine days of bait application, whereas none of the 18 radio-collared rats in the non-treatment plot died. There was a 99% drop in both the rat capture rate and percentage of non-toxic census bait blocks gnawed by rats in the treatment plot relative to the non-treatment plot three weeks after bait application. The only rat captured in the treatment plot three weeks after bait application was not ear-tagged (i.e., it was not a recapture), whereas 44% of the 52 rats captured in the non-treatment plot were ear-tagged. Most of the bait had disappeared from the forest floor within about one month of application. No birds likely to have eaten bait were found dead, although residues of diphacinone were found in the livers of three species of introduced seed-eating/omnivorous birds captured alive after bait application. No predatory birds were found dead one month or three months after bait application. The remains of a Hawaiian hawk (*Buteo solitarius*) were found six months after bait application, but it was not possible to determine the cause of death. This study demonstrated the efficacy of aerially broadcast diphacinone bait for control of rats and mice in Hawaiian montane forests, and was part of the dataset submitted to the U.S. Environmental Protection Agency for the national registration of a diphacinone bait for the control of rat populations in conservation areas.

## INTRODUCTION

Introduced black rats (*Rattus rattus*), Norway rats (*R. norvegicus*), and Polynesian rats (*R. exulans*) have been implicated in the decline or extinction of numerous species of plants and animals in Hawai'i and elsewhere in the Pacific (Atkinson 1977, 1985, Hadfield et al. 1993, Innes et al. 2010, Varnham 2010). Rodent control techniques used for conservation purposes in Hawai'i include snap-trapping, multi-kill devices, and the application of the first-generation anticoagulant diphacinone in bait stations. Snap-trapping and ground-based application in bait stations can be effective in small areas, but are labor-intensive and impractical for rodent control over large, remote, and rugged areas in Hawai'i. Aerial-broadcast application of bait containing vertebrate toxicants has been used successfully to control and eradicate introduced rodents for indigenous species conservation and ecosystem restoration over large areas in New Zealand (Innes et al. 1995, Towns and Broome 2003). Anticoagulant rodenticides have been aerially broadcast on off-shore islets in Hawai'i (Dunlevy and Swift 2010) and other Pacific islands (Howald et al. 2007, Engeman et al. 2013). This study addresses the efficacy of the aerial broadcast of diphacinone over large conservation areas of mesic habitat on Hawai`i Island, utilizing fragmented patches of older montane forests surrounded by younger lava flows: the Hawaiian kīpuka.

In a previous study, we demonstrated that hand-broadcast application of Ramik® Green bait containing 50 ppm (0.005%) diphacinone was effective in reducing populations of rats, predominantly black rats, in both wet and mesic montane forests in Hawai`i (Spurr et al. 2013). No non-target birds were found dead, although residues of diphacinone were found in the livers of three species of introduced birds captured alive. Based on the success of this and earlier

studies (Lindsey and Mosher 1994, Swift 1998, Dunlevy et al. 2000), we investigated the effectiveness against rats and mice, and risks to non-target species of aerial-broadcast application of 50 ppm diphacinone bait in mesic forests of Hawai'i Volcanoes National Park.

## METHODS

### Study Area

We used paired mesic forest sites (Figure 1) in Kīpuka Puaulu and Kīpuka Kī, two similar sized fragments of older (ca. 2,000 years old) deep ash-derived soils that support a tall-canopy forest and are separated by ca. 1 km of predominantly a'a lava of the late prehistoric Keamoku flows (ca. 450 years old) on the lower east slope of Mauna Loa between 1200–1360 m elevation (Mueller-Dombois and Lamoureux 1967). The kīpuka fall within a band of mesic habitat situated 3 km northwest of Kīlauea Caldera. Mature sparse 'ōhi'a trees (*Metrosideros polymorpha*) growing on the younger of these a'a flows have mostly died off in the last 15 years following a pattern of cohort senescence (Mueller-Dombois and Fosberg 1998), reducing the vegetation separating the sites. However, the National Park Service has been undertaking out-plantings and other restoration efforts focused on remnant open patches of deep ash soils and have greatly increased forest cover (primarily *Acacia koa*) in these areas, so the net effect in terms of isolation between these kīpuka is probably nil. We regard the open a'a lava as a natural barrier to dispersal for rats that inhibits movements between the two sites. Mueller-Dombois and Lamoureux ( 1967) reported that these kīpuka were each approximately 50 ha in size. More recent mapping efforts by the park and USGS indicates that the kīpuka are closer to 90–100 ha each. (Pratt et al. 2010). Ungulate control combined with out-planting over the last 40 years may have facilitated recovery as well as expansion of the kīpuka margins (Loh et al. 2009).

### Bait Application

An Experimental Use Permit issued by the U.S. Environmental Protection Agency for this study allowed only a single treatment plot of 50 ha. We treated the kīpuka as independent study sites and randomly assigned one as a treatment site (Kīpuka Kī) and the other as a non-treatment site (Kīpuka Puaulu). Because of permitting constraints, the study design was unreplicated with treatment and non-treatment plots approximately 1.5 km apart, in which rat populations were monitored before and after bait application. The plots were 675 × 675 m (45.56 ha), and were located in mesic forest on the lower slopes of Mauna Loa, Hawai'i Volcanoes National Park (HAVO), at an elevation of 1200–1360 m. A more complete description of the plots is given in Spurr et al. (2013).

The fauna within the study plots included eight endemic bird species — the Hawaiian Hawk or 'Io (*Buteo solitarius*), Hawaiian Short-Eared Owl or Pueo (*Asio flammeus sandwichensis*), 'Ōma'o (*Myadestes obscurus*), 'Elepaio (*Chasiempis sandwichensis*), 'Apapane (*Himatione sanguinea*), 'I'iwi (*Vestiaria coccinea*), and common 'Amakihi (*Hemignathus virens*). There was also one endemic mammal — the Hawaiian hoary bat or 'Ōpe'ape'a (*Lasiurus cinereus semotus*). Introduced bird species included the Barn Owl (*Tyto alba*), Kalij Pheasant (*Lophura leucomelana*), Northern Cardinal (*Cardinalis cardinalis*), Red-Billed Leiothrix (*Leiothrix lutea*), Melodious Laughing Thrush (*Garrulax canorus*), Japanese White-Eye (*Zosterops japonicus)*, and House Finch (*Carpodacus mexicanus*). Introduced mammals included the black rat, Norway rat, Polynesian rat, house mouse (*Mus musculus*), feral cat (*Felis catus*), and small Indian mongoose (*Herpestes auropunctatus*). The study was conducted in park management units with ungulate fences excluding feral pigs (*Sus scrofa*) and cattle (*Bos taurus*) (Tunison and

Stone, 1992). Historically, the dominant rodent in the study sites has been the black rat (Figure 2, Scheffler et al. 2012).

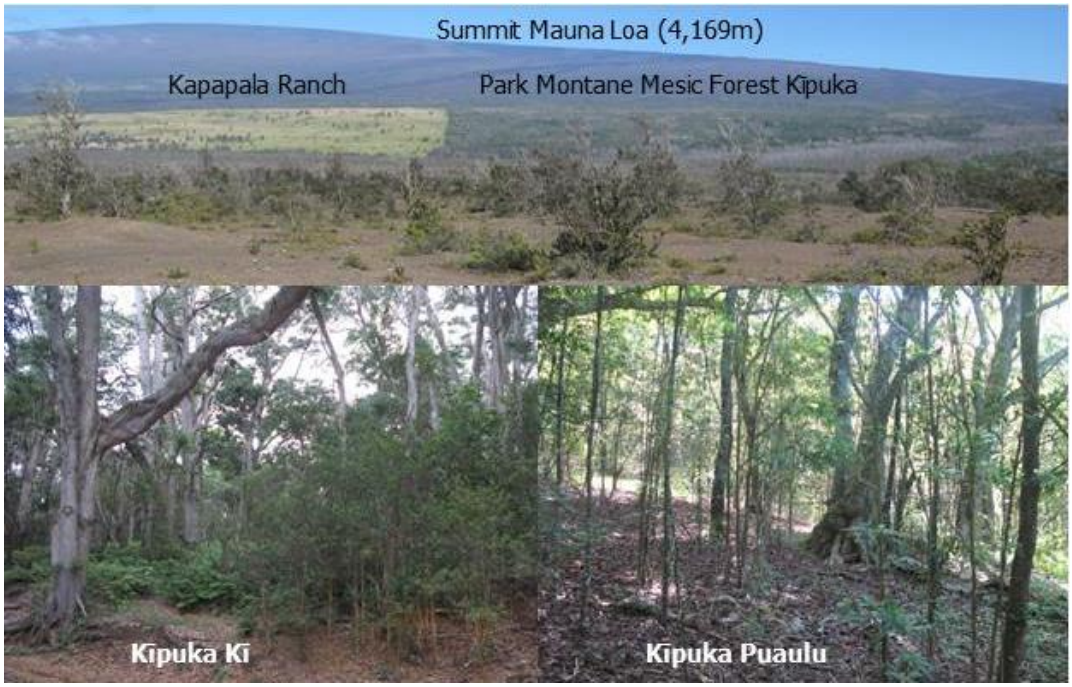


Figure 1. Panorama of montane mesic forest study sites on Mauna Loa Volcano looking North from the USGS Hawai'i Volcanoes Observatory showing belt of mesic forests to East interrupted by grasslands of Kapāpala Ranch to the West showing the forest-line park boundary. Examples of understories of Kīpuka Kī and Kīpuka Puaulu shown in insets below. (Photos: D. Foote, USGS)

A monitoring grid measuring 275 m × 275 m (7.56 ha) was established in the center of each study plot. The grid consisted of 12 transect lines 275 m long and 25 m apart. Each transect line was flagged with markers at 12.5-m intervals. Four of the transect lines extended 100 m beyond each end of the grid (for a total length of 475 m), and were used for searching for non-target mortality (see below).

The test bait was a fish-flavored, green-colored, pellet formulation of Ramik® Green (Lot No. 144548, HACCO, Inc., Madison, WI; Figure 2), nominally weighing 6 g and containing 50 ppm diphacinone. The bait was manufactured on 16 September 2000. The mean weight of 60 randomly selected pellets measured on 19 April 2001 was 6.29 g (± 0.06 g standard error) per pellet. The diphacinone concentration in a random sample of pellets, measured using high performance liquid chromatography (HPLC), was 51 ppm at the time of manufacture (HACCO, Inc., Madison, WI). This is within the Code of Federal Regulations certified limits of 45–55 ppm. It was 49.1 ± 0.5 ppm (mean ± standard error) in May 2001, when eight months old (Genesis Laboratories Inc., Wellington, CO), 52.0 ± 0.6 ppm in February 2002, when 17 months old (Genesis Laboratories Inc., Wellington, CO), and 50 ppm in May 2002, when 20 months old (HACCO, Inc., Madison, WI). The bait was 13 months old when applied to the treatment plot in October 2001.


Figure 2. Black rat (*Rattus rattus*) eating native snail (left); Ramik Green bait pellets (right) (Photos: Jack Jeffrey and HACCO, Inc., respectively)

The bait was applied from a specially designed helicopter bait bucket, imported from Lakeland Helicopters, Rotorua, New Zealand, slung under a Hughes 500D helicopter (Volcano Heli-Tours, Hilo, Hawai'i; Figure 3). The bucket consisted of a conical hopper with a 150-mm diameter opening in the bottom (that could be opened and closed with a gate) through which bait flowed onto a spinner that spread the bait over the treatment plot. The size of the opening could be varied by inserting rings with different-sized internal diameters into a socket in the bottom of the bucket. The size of the opening determined the rate of bait application because the helicopter flew at a constant height (approximately 45 m) and constant speed (approximately 111 km/h or 30.833 m/s). The correct size of opening to achieve a rate of application of 11.25 kg/ha of Ramik® Green bait was determined by a flow rate test (Spurr et al. unpublished data). As a result of this test, a wooden ring with an internal diameter of 128 mm was used in the first bait application on 25 October and a plastic ring with 124 mm internal diameter in the second bait application on 30 October.

The number of helicopter flights required to cover the treatment area was determined by measuring the swath width (the width of baits on the ground) in a trial aerial application of placebo Ramik® Green bait on 20 October 2001. As a result of this trial, seven helicopter flights were made over the 675-m wide treatment area, with an average swath width of 96.4 m (675 m wide / 7 flights = 96.4 m), for each toxic bait application.

The planned bait application rate was 22.5 kg/ha, in two applications of 11.25 kg/ha 5–7 days apart, the interval dependent upon suitable weather. A total of 635 kg of bait was applied on 25 October, and 481 kg on 30 October 2001. A mechanical malfunction of the bait bucket resulted in the unintentional application of some bait beyond the end of the flight paths, up to 30 m outside the treatment plot, on 25 October. Bait was also applied up to 80 m outside the treatment plot on 30 October. This illustrates the difficulty the pilot faces deciding when to close the gate in the bottom of the bait bucket. The rate of application within the treatment plot was estimated as 11.8 kg/ha on 25 October and 10.6 kg/ha on 30 October, a total of 22.4 kg/ha.

Figure 3. Loading bait into hopper (left) and preparing to spread bait above canopy of Kīpuka Kī (right) (Photos: D. Foote USGS).

The mean daily rainfall recorded in the treatment plot for five days after the first bait application (i.e., 26–30 October) was 3.7 mm, and for nine days after the second bait application (i.e., 31 October–8 November) was 1.0 mm. The average maximum and minimum daily temperatures for the five days after the first bait application were 73.0 °F and 51.7 °F, respectively. Temperatures were not recorded after the second bait application.

**Impact on Rat Abundance**

*Radio-telemetry*. – Radio-transmitters (Holohil PD-2C, weighing 4.2 g) were fitted to 25 black rats in the treatment plot and to 23 black rats in the non-treatment plot, one week before the first bait application. Four rats in the treatment plot and five rats in the non-treatment plot lost their transmitters before bait application, reducing the sample size to 21 rats in the treatment plot and 18 rats in the non-treatment plot. Radio-signals from the radio-collared rats were monitored as described by Spurr et al. (2013), nightly for three consecutive nights immediately before bait application, and for eight consecutive nights immediately after bait application, by which time all rats in the treatment plot were dead. The effectiveness of bait application was

determined statistically using a 2 × 2 Chi-square analysis of the number of radio-collared rats alive vs. dead in the treatment and non-treatment plots before and after bait application, as described by Spurr et al. (2013).

*Live-trapping*. – A total of 144 Haguruma® wire-cage traps were placed at 25-m intervals on transect lines spaced 25 m apart within each study plot. The traps were left closed for at least two weeks before the first trapping to allow the rats time to become accustomed to the traps. The traps remained at the trap locations throughout the study period. Two weeks before bait application in the treatment plot, trap locations were pre-baited with shredded coconut for three nights, and then the traps were opened and baited with coconut chunks for four consecutive nights (maximum 576 trap-nights). The traps were checked daily, and all rats that did not escape were identified to species, sex, and age class (juvenile or adult), weighed, ear-tagged, and then released. Traps were operated again as above three weeks, three months, and six months after bait application to determine efficacy and rat reinvasion rates. Rat captures per 100 corrected trap-nights were calculated following the method of Nelson and Clark (1973).

The percentage reduction in rat capture rates in the treatment plot, relative to the non-treatment plot, was calculated from the formula:

% reduction = 100 × ((expected capture rate – observed capture rate) / (expected capture rate)) ..... (1),

where expected capture rate = capture rate in treatment plot pre-treatment × (capture rate in non-treatment plot post-treatment / capture rate in non-treatment plot pre-treatment), and observed capture rate = capture rate in treatment plot post-treatment.

It was not possible to statistically compare rat capture rates per 100 corrected trap-nights in the treatment and non-treatment plots before and after bait application, to assess the effectiveness of bait application, because there was no replication of plots (only pseudo-replication of traps). Chi-square analysis could not be used on the raw data (number of rats caught) because the analysis could not take into account the different number of trap-nights in each trapping session, resulting from different numbers of rats and non-target species (house mice, mongooses, and birds) that were caught, nor could it allow for spatial or temporal correlation between trappings.

*Non-toxic census bait blocks*. – A total of 132 non-toxic cereal and wax-based Census™ bait blocks (Zeneca Professional Products, Wilmington, DE) were placed at 25-m intervals on the same transect lines as live-traps in each study plot, but half-way between the live-trap locations, two weeks before and one month, three months, and six months after bait application. They were attached to the ground using a 1-m wire flag inserted through a hole in the center of each block. The blocks were examined daily for two consecutive days (maximum 264 bait nights) for signs of feeding by rats or other animals (viz., house mice, mongooses, birds, and invertebrates). Unfortunately, up to 64% of the blocks went missing between checks. It was not possible to determine which animal species was removing the blocks, so rat interference was calculated on those blocks remaining. Because there was unlikely to have been a linear relationship between the proportion of blocks interfered with and rat density (Caughley 1977, Spurr 1995), the data were log-transformed according to the equation:

Rat density index (rats/block) = $-\log_e (1-f)$ ..... (2),

where $f$ = the proportion of blocks interfered with.

The percentage reduction in the number of census blocks gnawed by rats in the treatment plot, relative to the non-treatment plot, was calculated in the same way as for the live-trapping data. It was not possible to statistically compare rat gnawing on census blocks in the treatment and non-treatment plots before and after bait application for the same reasons as for live trapping (viz., because there was no replication of plots).

**Impact on Mouse Abundance**

*Kill trapping*. – Fifty-six Victor® mouse snap-traps were located at 10-m intervals (two traps per location) along one transect line in each study plot to estimate mouse densities pre- and post-treatment. Trapping was carried out three weeks before, and one month, three months, and six months after bait application. Within each trapping session, the traps were baited with coconut chunks, set, and examined daily for two consecutive days (maximum 112 trap nights). The traps were removed after a trapping session, and re-located to a new transect for the next trapping session. Mouse capture-rates pre- and post-treatment in the treatment and non-treatment plots were calculated in the same way as for rats.

*Live trapping*. – Mice were caught in the live-traps used for monitoring rats, and mouse capture rates were calculated in the same way as for rats.

*Non-toxic census bait blocks*. – Mice left distinctive gnaw-marks on the census bait blocks used for monitoring rats, and the level of mouse interference to blocks was calculated in the same way as for rats.

**Non-target Species Risk Assessment**

*Risk from baits*. – The duration for which baits were present on the forest floor, exposed to non-target as well as target species, was monitored after both the first and second bait applications. Individual baits were placed beside colored wire flags at 20 randomly selected locations (at least 25 m apart) along transects in one quarter of the central monitoring area. The presence or absence of each bait was recorded daily for 14 days or until the bait disappeared or disintegrated.

*Risk from dead animals*. – The locations of radio-collared rats and other animals found dying or dead during the study were recorded to determine if the carcasses were exposed to avian predators (viz. hawks and owls). Any carcasses that could be collected were necropsied and examined for green bait in the stomach and intestines, and for hemorrhaging characteristic of anticoagulant poisoning. The carcasses were then frozen and sent to Genesis Laboratories Inc. (Wellington, CO) or Landcare Research (Lincoln, New Zealand) for HPLC determination of diphacinone residues in their livers.

*Dead birds*. – Four randomly selected strip transects (475 m long by 5 m wide), spaced at least 25 m apart, in the treatment and non-treatment plots (representing 2% of each plot), were walked to search for non-target mortality on 15 October (2 weeks before bait application), and 13 November, 11 February, and 13 May (3 weeks, 3 months, and 6 months after bait application). The searching took about three person-hours on each sampling occasion. In addition, at least 500 person-hours were spent in each plot carrying out other activities (such as live-trapping, radio-telemetry, and census bait block monitoring) on each sampling occasion. All

carcasses found were recorded as to species, weight, and sex, frozen, and then sent for determination of diphacinone residues in their livers (as above).

*Live birds*. – Avian predators (viz. hawks and owls) present in the study areas pre- and post-treatment were noted whenever they were observed. An index of abundance of Kalij Pheasant was potentially available from the frequency of pecking at the non-toxic census bait blocks used for monitoring rats. However, while Kalij Pheasants pecked at bait blocks in the non-treatment area (where they were abundant); none did so in the treatment area (where they were scarce).

Birds of four introduced species (viz., Kalij Pheasant, Red-Billed Leiothrix, Northern Cardinal, and Japanese White-Eye) were collected by shooting or mist-netting in the treatment plot one month after bait application. Birds (viz., Red-Billed Leiothrix) accidentally caught in kill-traps set for mice were also collected to increase sample sizes. The carcasses were frozen and sent for determination of diphacinone residues in their livers (as above).

*Invertebrates*. – Invertebrates found alive on and/or immediately underneath Ramik® Green baits were identified (to species where possible) and counted at 1–4-day intervals from 26 October (one day after the first bait application) to 16 November 2001. Baits were placed at 2.5-m intervals beside wire flags on four transect lines, of 25 baits per line, on 25 October and on another four lines on 30 October 2001. Observations were made during the day, between 0930 and 1300 hours, and at night, using a headlamp, between 1800 and 2000 hours (shortly after sunset) and between 0400 and 0600 hours (shortly before sunrise). Invertebrate samples were collected from baits, pooled into 1-g amounts by species, frozen, and sent to Landcare Research (Lincoln, New Zealand) for determination of diphacinone residues in the whole body. Invertebrate samples were also collected in dry pitfall traps during the week before, and one week, one month, and three months after bait application, and analyzed for diphacinone residues as above. The pitfall traps were set at 12.5-m intervals on four randomly selected transect lines.

## RESULTS

### Impact on Rat Abundance

*Radio-telemetry*
All 21 radio-collared black rats in the treatment plot died within nine days of the initial bait application on 25 October 2001. None of the 18 radio-collared black rats in the non-treatment plot died in that time. Post-treatment survival of the radio-collared rats in the treatment plot (0 of 21 rats) and non-treatment plot (18 of 18 rats) was significantly different ($\chi^2 = 39.0$, df = 1, $P < 0.001$).

*Live-trapping*
One week before bait application, 82 black rats, one Polynesian rat, and one Norway rat were captured in the treatment plot, and 74 black rats in the non-treatment plot. Three weeks after bait application, however, just one black rat was caught in the treatment plot whereas 50 black rats and two Polynesian rats were caught in the non-treatment plot. This represents a 98.5% drop in the rat capture rate (per 100 corrected trap nights) in the treatment plot relative to the non-treatment plot three weeks after bait application (Table 1). Three months after bait application, 14 black rats were caught in the treatment plot and 22 black rats in the non-treatment plot. Six months after bait application, 11 black rats and one Polynesian rat were

caught in the treatment plot, and 15 black rats and one Polynesian rat in the non-treatment plot. Thus, the rat capture rate was still reduced in the treatment plot relative to the non-treatment plot six months after bait application (Table 1).

Table 1. Rat captures (per 100 corrected trap nights) in live-traps before and after aerial application of Ramik® Green bait.

| Time | Non-treatment plot | Treatment plot | % Change in treatment plot after treatment |
|---|---|---|---|
| Before (16–19 Oct 2001) | 15.4 | 20.0 | |
| After 3 weeks (14–17 Nov 2001) | 10.1 | 0.2 | –98.5 |
| After 3 months (12–15 Feb 2002) | 4.2 | 2.8 | –48.7 |
| After 6 months (7–10 May 2002) | 2.9 | 2.4 | –36.3 |

The one rat captured in the treatment plot in November, three weeks after the initial bait application in October, was not ear-tagged (i.e., it was not a recapture from before bait application). Likewise, of the 14 rats captured in February, three months after bait application, and 12 rats captured in April, six months after bait application, none had been captured and ear-tagged before bait application. In contrast, in the non-treatment plot, 44% of the 52 rats captured in November, 55% of the 22 rats captured in February, and 44% of the 16 rats captured in April had been captured and ear-tagged in October before bait application.

Proportionately more males and proportionately more juveniles were caught in the treatment plot than in the non-treatment plot in the first six months after bait application (Table 2). However, most of the rats caught in the treatment plot after bait application were adult males. This may reflect the time of year (late October- early November) that the trial poisoning operation was carried out. The rat capture rate in the non-treatment plot declined naturally from October 2001 to May 2002, presumably as a result of no or little breeding or recruitment over winter. Thus, there may have been fewer juveniles available to re-colonize the treatment plot than if the trial poisoning operation had been done in spring or summer.

Table 2. Sex and age classes of rats caught before and after aerial application of Ramik® Green bait (Sample sizes differ from numbers captured because some rats escaped before they could be sexed or aged).

| Time | Sex ratio | | | | Age ratio | | | |
|---|---|---|---|---|---|---|---|---|
| | Non-treatment | | Treatment | | Non-treatment | | Treatment | |
| | M : F | (n) | M : F | (n) | Ad:Juv | (n) | Ad:Juv | (n) |
| Before (16–19 Oct 01) | 60:40 | (65) | 54:46 | (79) | 52:48 | (65) | 40:60 | (79) |
| After 3 weeks (14–17 Nov 01) | 53:47 | (47) | 100:0 | (1) | 66:34 | (47) | 0:100 | (1) |
| After 3 months (12–15 Feb 02) | 55:45 | (22) | 77:23 | (13) | 95:5 | (22) | 54:46 | (13) |
| After 6 months (7–10 May 02) | 46:54 | (13) | 80:20 | (10) | 100:0 | (13) | 90:10 | (10) |

M = male, F = female, Ad = adult, Juv = juvenile.

*Non-toxic census bait blocks*

Rat gnawing on remaining census bait blocks was reduced by 99.2% in the treatment plot relative to the non-treatment plot three weeks after bait application, and was still reduced in the treatment plot 6 months after bait application (Table 3). In addition, the incidence of missing census bait blocks was reduced by 98.2% in the treatment plot relative to the non-treatment plot three weeks after bait application, and also was reduced by a similar amount as gnawed blocks in the treatment plot six months after bait application. This indicates that rats were responsible for removing the missing blocks.

Table 3. Rat density indices from interference to census bait blocks before and after aerial application of Ramik® Green bait.

| Time | Non-treatment plot | Treatment plot | % Change in treatment plot after treatment |
|---|---|---|---|
| Before (11–12 Oct 2001) | 0.63 | 0.90 | |
| After 7 weeks (5–6 Dec 2001) | 0.72 | 0.008 | –99.2 |
| After 3 months (6–7 Feb 2002) | 1.04 | 0.21 | –85.9 |
| After 6 months (2–3 May 2002) | 0.64 | 0.21 | –77.0 |

**Impact on Mouse Abundance**

*Kill-trapping*
The number of mice caught in snap-traps was reduced by 78.9% in the treatment plot relative to the non-treatment plot three weeks after bait application (Table 4). The one mouse captured in the treatment plot three weeks after bait application contained 3.8 ppm diphacinone in its liver. Mouse captures recovered to near pre-poison levels three months after bait application, and were almost three times pre-poison levels six months after bait application.

Table 4. Mouse captures (per 100 corrected trap-nights) in snap-traps before and after aerial application of Ramik® Green bait.

| Time | Non-treatment plot | Treatment plot | % Change in treatment plot after treatment |
|---|---|---|---|
| Before (2–3 Oct 2001) | 1.19 | 4.46 | |
| After 1 month (29–30 Nov 2001) | 1.09 | 0.86 | –78.9 |
| After 3 months (24–25 Jan 2002) | 2.17 | 5.00 | –38.5 |
| After 6 months (25–26 Apr 2002) | 0.96 | 12.50 | +247.4 |

*Live-trapping*
The number of mice caught in live traps was reduced by 75.6% in the treatment plot relative to the non-treatment plot three weeks after bait application (Table 5). Two of the mice captured in the treatment plot three weeks after bait application died in the traps. They contained 0.42 and 1.3 ppm diphacinone in their livers. Mouse captures recovered to near pre-poison levels three months after bait application.

Table 5. Mouse captures (per 100 corrected trap-nights) in live-traps before and after aerial application of Ramik® Green bait.

| Time | Non-treatment plot | Treatment plot | % Change in treatment plot after treatment |
|---|---|---|---|
| Before (16–19 Oct 2001) | 4.1 | 11.2 | |
| After 3 weeks (14–17 Nov 2001) | 2.1 | 1.4 | –75.6 |
| After 3 months (12–15 Feb 2002) | 2.1 | 8.0 | +39.5 |
| After 6 months (7–10 May 2002) | 0.4 | 8.1 | +641.3 |

*Non-toxic census bait blocks*

Mouse gnawing on census bait blocks was reduced by 95.0% in the treatment plot relative to the non-treatment plot one month after bait application (Table 6). If the large increase in the non-treatment plot is ignored, the decrease in the treatment plot was only 70.6%. Mouse gnawing on census bait blocks in the treatment plot increased to about two-thirds of the pre-poison level three months after bait application.

Table 6. Mouse density indices from interference to census bait blocks before and after aerial application of Ramik® Green bait.

| | Non-treatment plot | Treatment plot | % Change in treatment plot after treatment |
|---|---|---|---|
| Before (11–12 Oct 2001) | 0.07 | 0.36 | |
| 1 month after (5–6 Dec 2001) | 0.38 | 0.11 | –95.0 |
| 3 months after (6–7 Feb 2002) | 0.08 | 0.25 | –41.4 |
| 6 months after (2–3 May 2002) | 0.14 | 0.24 | –71.5 |

**Non-target Species Risk Assessment**

*Risk from baits*

The disappearance rate of baits was more rapid after the first application on 25 October than after the second application on 30 October (Figure 4), perhaps as a result of higher rat numbers. However, a similar percentage of monitored baits (25%) remained two weeks after each application. From incidental observations, most baits had disappeared one month after application. However, the remains of at least one bait were still present on the forest floor on 25 January 2002, three months after application. Some remaining baits (number not quantified) had signs of feeding by slugs (smooth, slimy hollows on the surface) and other invertebrates, probably beetles (small particles of bait around the main piece of remaining bait). (cf. Johnston et al. 2005)

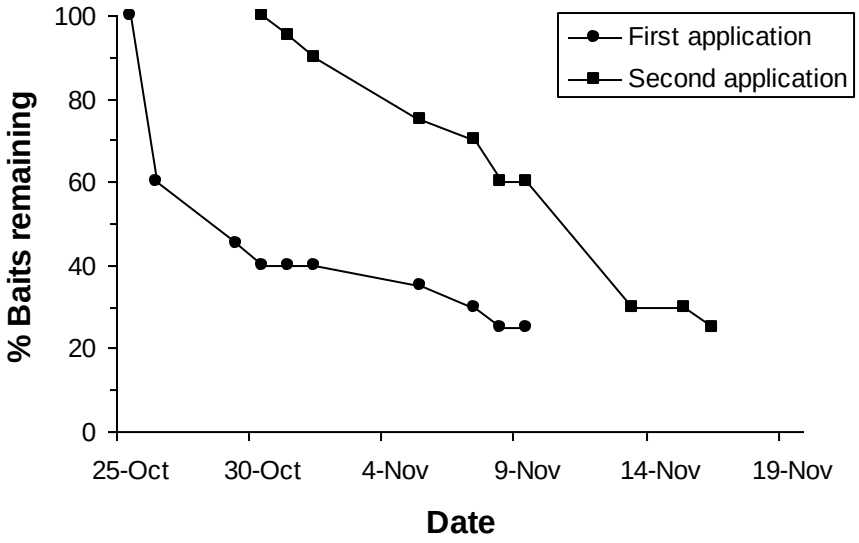


Figure 4. Disappearance rate of Ramik® Green bait (expressed as % baits remaining on the forest floor) after aerial application, Kīpuka Kī, Hawai'i Volcanoes National Park, 25 and 30 October 2001.

*Risk from dead animals*

Six radio-collared rat carcasses were found after bait application, and all were in locations inaccessible to avian predators, viz., underground or in dense vegetation. One other radio-collared rat, found four days after the first bait application, barely alive, bleeding from the ventrum and stumbling along on a fallen branch during the daytime, was clearly exposed to avian predators. Its radio-collar was recovered later on the ground surface without a carcass, which presumably had been scavenged by a mammalian predator, perhaps a cat or a mongoose. Eight other radio-collars were recovered without carcasses, which again presumably had been scavenged by mammalian predators. These were all underground or in dense vegetation and, on the basis of where other carcasses were recovered, it is presumed they died there. Seven radio-collared rats and their collars were unable to be recovered because they were under immovable rock, deep in hollow old trees, or high up in the canopy. Thus, of the 21 radio-collared rats, 20 (95%) were considered to have died in locations inaccessible to avian predators, and only one (5%) in a location accessible to avian predators.

In addition to the carcasses of the six radio-collared rats, two other black rat carcasses, including an ear-tagged one, were found in the treatment plot after bait application. Necropsies of the eight rats revealed indications of diphacinone poisoning (Table 7). All had internal hemorrhaging (under skin, around heart, and in lungs, liver, bladder, genitals, thoracic cavity, and abdominal cavity) and five also had external hemorrhaging (from mouth, nose, ear, anus, and genital region). Five of the rats also had green bait in their stomachs and/or green fecal pellets in their intestines. An average of 4.4 ppm diphacinone was detected in the livers of those analyzed (Table 7). One rat had a liver residue value of 12 ppm, which is the highest liver residue value reported for diphacinone (Eisemann and Swift 2006, Rattner et al. 2011).

Table 7. Signs of diphacinone poisoning in rats found dead after aerial application of Ramik® Green bait (Rat 3 was not tested for diphacinone residues because it was too decomposed).

| Rat number | Internal bleeding | External bleeding | Green dye in gut | Diphacinone (ppm) in liver |
|---|---|---|---|---|
| 1 | + | + | + | 2.4 |
| 2 | + | + | + | 6.5 |
| 3 | + | + | | – |
| 4 | + | + | | 12.0 |
| 5 | + | | + | 4.1 |
| 6 | + | | + | <MDL |
| 7 | + | + | | 3.5 |
| 8 | + | | + | 2.1 |
| Total | 8/8 | 5/8 | 5/8 | Average 4.4 |

<MDL = less than the Method Detection Limit of 0.1 µg/g for a 2 g sample (Landcare Research)

In addition to the dead rats, three dead mice were found in the treatment plot. Two were found six and seven days after the first bait application, on the ground surface, exposed to avian predators. They contained 2.1 and 2.4 ppm diphacinone in their livers. The third dead mouse was found nine days after the first bait application, in a nest under a log inaccessible to avian predators. The diphacinone concentration in its liver was not determined.

*Dead birds*

No dead birds were found during any of the ground searches in the treatment or non-treatment plots (in about three person-hours, covering 2% of each plot). However, the remains of a dead Hawaiian hawk (feathers, beak, and legs) were found in the treatment plot on 9 May 2002, six months after bait application, during extensive visits for radio-telemetry, live-trapping, and census bait block surveys (of at least 500 person-hours). There was no evidence to indicate whether the bird was an adult or a juvenile, or how it died, and the remains were unsuitable for diphacinone analysis.

*Live birds*

A pair of Hawaiian hawks was present in the treatment plot throughout the study. They had a juvenile with them at the time of bait application in October 2001. The fate of the juvenile is not known, but the pair was still present one month after bait application. One Hawaiian hawk was seen occasionally in the non-treatment plot throughout the study. No Hawaiian short-eared owls or barn owls were seen in either the treatment or non-treatment plots.

Residues of diphacinone were detected in the livers of all Kalij Pheasant, Red-Billed Leiothrix, and Northern Cardinal but not in any Japanese White-Eye captured alive in the treatment plot 1 month after bait application (Table 8). The highest residue level (4.9 ppm) was found in a Red-Billed Leiothrix. The sample sizes were too small to be certain of the true proportion of the bird populations that had ingested diphacinone, and too small to say that no Japanese White-Eye had ingested diphacinone.

Table 8. Diphacinone residues in the livers of non-target bird species collected alive 1 month after aerial application of Ramik® Green bait.

| | Kalij Pheasant | Red-billed Leiothrix | Northern Cardinal | Japanese White-eye |
|---|---|---|---|---|
| Number sampled | 2 | 8 | 2 | 5 |
| % positive diphacinone | 100 | 100 | 100 | <MLD |
| Mean ppm diphacinone | 0.15 | 2.45 | 0.11 | <MLD |
| Range ppm diphacinone | 0.12–0.18 | 0.74–4.9 | 0.08–0.13 | <MLD |

<MDL = less than the Method Detection Limit of 0.1 µg/g for a 2 g sample (Landcare Research)

*Invertebrates*

At least 35 species of invertebrates were observed on and/or immediately under Ramik® Green baits (Table 9). The most common were Collembola (possibly more than one species), a large black Carabid beetle (*Laemostenus complanatus*), and the Garlic snail (*Oxychilus alliarius*) (Figure 5). Carabid beetles formed the greatest biomass on baits, especially at night, and reduced baits to crumbs in a matter of days. Garlic snails and slugs (*Deroceras* spp., *Limax maximus*, and *Milax gagates*) also actively fed on baits, and green bait particles were found in their gut and feces. Slugs characteristically left smooth, slimy hollows on the surface of baits.

Table 9. Invertebrate species observed on and/or immediately under Ramik® Green bait, Hawai'i Volcanoes National Park.

| Class | Order | Family | Genus and species |
|---|---|---|---|
| Oligochaeta | | | Earthworm sp. |
| Gastropoda | Stylommatophora | Zonitidae | *Oxychilus alliarius* (garlic snail) |
| | | Agriolimacidae | *Deroceras laeve* |
| | | Agriolimacidae | *Deroceras reticulatum* |
| | | Agriolimacidae | *Deroceras* sp. |
| | | Limacidae | *Limax maximus* |
| | | Milacidae | *Milax gagates* |
| Turbellaria | Tricladida | | Flatworm sp. |
| Crustacea | Isopoda | Porcellionidae | *Porcellio scaber* |
| | Amphipoda | | Amphipod sp. |
| Arachnida | Araneida | | Spider sp. |
| | Acarina | | Mite sp. |
| Myriapoda | Diplopoda | | Large millipede sp. |
| | | | Small millipede sp. |
| | Chilopoda | | Centipede sp. |
| Collembola | Collembola | Entomobryidae | Springtail sp. |
| Insecta | Dermaptera | Forficulidae? | Earwig sp. |
| | Orthoptera | Acrididae | Grasshopper sp. |
| | | Gryllidae | Cricket sp. |
| | Hemiptera | Lygaeidae | Seed bug sp. |
| | Coleoptera | Carabidae | *Laemostenus complanatus* |
| | | Carabidae | Small brown beetle sp. |
| | | Carabidae | Medium brown beetle sp. |
| | | Elateridae | Click beetle sp. |
| | | Nitidulidae | Sap beetle sp. |
| | | Staphylinidae | Rove beetle sp. |

| | | |
|---|---|---|
| Diptera | Tipulidae | Crane fly sp. |
| | Drosophilidae | Pomace fly sp. |
| | Phoridae | Phorid sp. |
| | ??? | Fly sp. |
| Lepidoptera | | Lepidopteran sp. (larva) |
| | | Lepidopteran sp. (larva) |
| | | Lepidopteran sp. (larva) |
| Hymenoptera | Formicidae | Ant sp. |
| Psocoptera | Psocidae | Bark louse sp. |

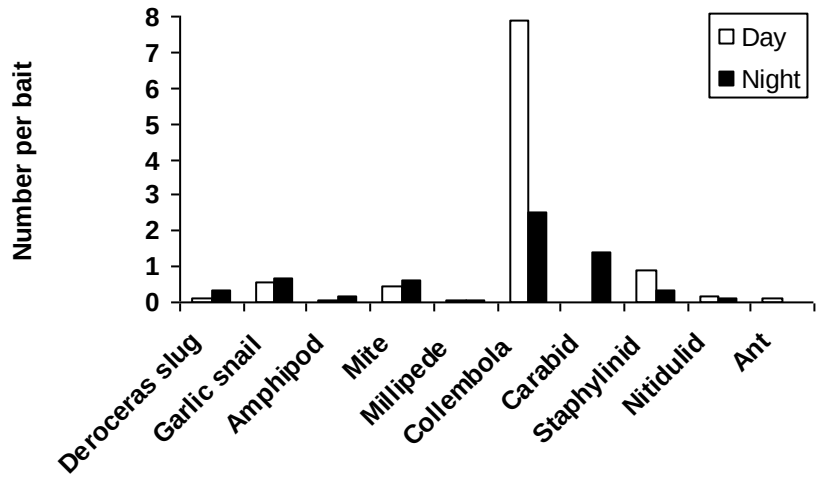


Figure 5. Invertebrates observed on Ramik® Green baits, Kīpuka Kī, Hawai'i Volcanoes National Park, October – November 2001.

The invertebrates collected from baits contained high concentrations of diphacinone, up to 3.7 ppm (Table 10). The invertebrates collected in pitfall traps up to one week, one month, and three months after aerial application of baits also contained high concentrations of diphacinone, up to 4.0 ppm.

Table 10. Diphacinone residues (ppm) in invertebrates collected after aerial application of Ramik® Green bait (<LD is below limit of detection).

| Invertebrate | Time after application | | | |
|---|---|---|---|---|
| Taxa | On baits | <1 week | 1 month | 3 months |
| Garlic snail (*O. alliarius*) | 3.4 | 2.2 | 4.0 | 1.8 |
| Slug (*Deroceras* sp.) | 3.7 | 1.4 | 1.7 | 1.1 |
| Slug (*Limax maximus*) | 3.7 | 1.3 | 3.1 | 2.0 |
| Isopod (*Porcellio scaber*) | – | 0.3 | 0.3 | – |
| Millipede | 0.6 | – | – | <LD |
| Carabid (*L. complanatus*) | 2.8 | 1.7 | 1.9 | <LD |
| Lepidopteran larva | 2.3 | 0.6 | <LD | 0.3 |

## DISCUSSION

Aerial-broadcast application of Ramik® Green bait containing 50 ppm diphacinone, at about 22.5 kg/ha (in two applications of about half this amount, one week apart), was followed by 100% mortality of rats based on radio-telemetry results and 99% mortality of rats based on live-trapping and non-toxic census bait block interference results. The synchronous death of rats following bait application, together with the presence of bait in their stomachs, bleeding characteristic of anticoagulant poisoning, and diphacinone residues in their livers, indicate that the rats likely died of diphacinone poisoning. The rats captured in this study were predominantly black rats. Insufficient Polynesian and Norway rats were captured to determine the effect of aerial application of Ramik® Green bait on their population numbers. The results of this study, as with those of our previous study (Spurr et al. 2013), demonstrate that broadcast application of Ramik® Green bait is highly effective in reducing populations of black rats in forest habitat in Hawai'i.

House mouse numbers were reduced by the aerial application of 50 ppm diphacinone bait in this study, but not by as much as rat numbers. The $LD_{50}$ of diphacinone for mice (50–300 mg/kg) is higher than for rats (0.3–7 mg/kg), and mice also have smaller home ranges than rats, reducing the likelihood that all mice would have encountered sufficient bait for lethal doses in this study. The large size of the pellets (over 6 g) compared to the size of a mouse (8–16 g; Tomich 1986) would also have discouraged mice from foraging on the pellets. The rebound in abundance prior to the recovery of rats is consistent with the increases in densities of mice documented following rat removal in other studies (Witmer et al. 2007, Harper and Cabrera 2009, Ruscoe et al. 2011). As noted by Spurr et al. (2013), the different methods of monitoring the efficacy of toxic bait application had different strengths and weaknesses. Radio-telemetry was the best method, because it enabled the fate of known individuals to be determined. Live-trapping, with ear-tagging, was also useful but it was not possible to be certain whether the rat without ear-tags captured in the treatment plot three weeks after bait application was present

before bait application and survived (by not encountering baits, encountering baits but not eating them, or eating insufficient bait), or whether it had only moved into the treatment plot after bait application. The length of time between bait application and live-trapping (3 weeks), the ability of rats to move over distances of several hundred meters within this period of time, and the fact that 44% of the rats captured in the non-treatment plot were recaptures supports the interpretation that it was more likely to be an immigrant rather than a survivor of the treatment. Interference to non-toxic census bait blocks was the most difficult method to interpret because of the difficulty of deciding which species (rats, mice, mongooses, birds, or invertebrates) had interfered with the census blocks and deciding what to do about missing census blocks (Spurr et al. 2013). In this study, missing census blocks were deleted from the calculations, and this could have exaggerated the percentage interference to the remaining blocks by less common species. Nevertheless, the large reduction in missing bait blocks and in rat interference to the remaining bait blocks after bait application indicates a large reduction in rat numbers.

The length of time that bait remained on the forest floor does not appear to have presented a hazard to birds from direct consumption of bait because no mortality of birds likely to have eaten bait was observed. The presence of diphacinone residues in the livers of Kalij Pheasant (up to 0.18 ppm), Red-Billed Leiothrix (up to 4.9 ppm), and Northern Cardinal (up to 0.13 ppm), all primarily seed-eating and/or omnivorous birds, suggests that they had eaten bait. Bird species previously observed eating Ramik® Green baits in Hawai'i are the Kalij Pheasant (Spurr et al. 2013), Erckel's Francolin (*Francolinus erckelli*), Japanese Bush Warbler (*Cettia diphone*), and Red-Billed Leiothrix (P. Dunlevy, unpublished data). There is no known relationship between liver concentration and lethal dose for diphacinone in birds (Erikson and Urban 2004), and lethal doses as determined in laboratory studies vary widely among species and between individuals within the same species (Rattner et al 2012). Therefore, the data from realistic exposure scenarios such as this study are the best predictor of risk from future broadcast applications.

The risk of secondary poisoning of predatory birds (viz. the Hawaiian hawk, Hawaiian short-eared owl, and barn owl) from dead rat carcasses was low, because 95% of rats died in inaccessible locations. Lindsey and Mosher (1994) reported that avian predators did not take any of the dead (kill-trapped) rats that they placed on the forest floor, but non-native mammalian predators rapidly found and consumed them. Lindsey and Mosher (1994) also reported that radio-collared rats moving during the day, before and after consuming diphacinone bait, remained under cover, minimizing their exposure to avian predators. However, one radio-collared diphacinone-poisoned rat in our study was found moving on the forest floor accessible to avian predators during the day. This, along with mice that died in the open, demonstrates that live rodents that have not yet succumbed to the poison's effects do pose a risk to raptors. Furthermore, residue levels in one of the mice and one of the rats were above theoretical thresholds calculated for mortality and sublethal effects to the Hawaiian hawk and Hawaiian short-eared owl if they consumed rodents over multiple days (Rattner et al. 2011). However, no conclusions about the actual risk to raptors from an aerial broadcast of diphacinone can be drawn from this study. The Hawaiian hawk that was found dead in the treatment plot in our study was not found until 6 months after bait application, despite extensive visits to the treatment plot one month and three months after bait application, and there was insufficient evidence left to indicate how it died.

The risk of secondary poisoning of insectivorous non-target species (birds and bats) from ingestion of invertebrates that have eaten baits was not assessed directly in our study. No insectivorous birds or bats were found dead. Invertebrates that eat Ramik® Green baits are unlikely to be killed by diphacinone because they do not have the same blood clotting systems as vertebrates (Shirer 1992), but do retain the diphacinone in their tissues. By checking baits at night as well as during the day, we found a larger range of invertebrates on baits than Dunlevy et al. (2000) found during the day. Some species occurred only at night. Invertebrates that have eaten baits are more likely to form part of the diet of non-target species that scavenge in the forest litter rather than of those that feed from tree trunks or in the canopy. As well as posing a potential risk of secondary poisoning to insectivorous non-target species, invertebrate consumption of baits also reduces bait availability to rats. However, the extent of this consumption was not sufficient to affect the efficacy of the bait application for rat control in our study. The high levels of diphacinone found in invertebrates up to at least three months after aerial application of bait is disconcerting. We are not aware of any previous reports of the long-term persistence of diphacinone in invertebrates. However, Johnston et al. (2005), reported short-term diphacinone persistence in three species of Hawaiian introduced gastropods (*Oxychilus* spp.*, Deroceras laeve,* and *Limax maximus*) fed ad libitum for seven days on Ramik® Green pellets. The diphacinone residue levels at seven days post-exposure did not decline from the levels found at 0 hours and 24 hours of the post-exposure period. It is plausible that some pellets persisted following the knock down of rat populations and provided a source of diphacinone for invertebrates over a longer time-frame. The disappearance rates of bait pellets in Hawaiian forests following the removal of rats needs further study.

Rat populations reduced by rodenticides normally recover rapidly by invasion of new rats, and subsequent reproduction, within 3–12 months, depending upon the size and shape of the treated area (Innes et al. 1995, Nelson et al. 2002, Spurr et al. 2013). Consequently, for consistent reduction in rat numbers, rodenticides need to be applied continuously or periodically after the first application. It may be possible to increase the breeding success of some species of birds with annual application of baits for rat control during the peak bird breeding season, say February for Hawai'i 'Elepaio (Sarr et al 1998) or April–July, as suggested for indigenous species protection from rat predation in New Zealand forests (Innes et al. 1995). Protection of native plants and invertebrates from rats and mice, as well as ecosystem-level restoration, could be achieved if sufficiently large areas are treated repeatedly.

## Management Implications

The results of this study, as with those of our previous study (Spurr et al. 2013), demonstrate that broadcast application of Ramik® Green bait is highly effective in reducing populations of black rats and mice in forest habitat in Hawai'i. Black rats are the dominant rodent and predominant predator in forest bird habitat in Hawai'i (Lindsey et al. 2009, Scheffler et al 2012). If bait application was timed to coincide with the beginning of the breeding season of birds, say April, as suggested for indigenous species protection from rat predation in New Zealand forests (Innes et al. 1995, Powlesland et al. 2003), it may provide a window of opportunity of several months for birds to successfully nest, forage and fledge young. The high rate of nest failure observed in the primarily insectivorous Hawai'i Elepaio (Sarr et al. 1998) in areas where invasive small mammals are common (Scheffler et al. 2012) suggests the need for both protection from direct nest predation and from resource competition of invasive rodents. Hawai'i Volcanoes National Park utilizes Special Ecological Areas for alien plant management (Tunison and Stone 1992). A similar management approach has been used in New Zealand for habitat restoration

utilizing the "mainland island" concept (Saunders 2000). There, extensive research has been conducted to develop management tools that target local reductions of selected pest species to below threshold levels where adverse impacts are observed on a variety of vulnerable native species, all within an ecosystem focus. The efficacy and relative safety of diphacinone baits for suppression of rat and mouse populations in Hawaiian forests demonstrates that this is a potentially strong management tool that can be applied within focal management areas for the benefit of Hawai'i's endemic species.

## ACKNOWLEDGEMENTS

This study was initiated by the late Gerald Lindsey. Assistance with planning was given by E. Campbell, L. W. Pratt, R. Sugihara, C. Swift, and T. Tunison. We are grateful to the National Park Service for permission to undertake the study in Hawai'i Volcanoes National Park; T. Casey and P. Simmons, Kamehameha Schools, for permission to undertake the placebo bait trial on Keauhou Ranch; D. Morgan for advice on determining flow rate and swath width of the baits; J. Bazzano, I. Cooper, M. Dean, P. Dunlevy, W. Erb, L. Gold, D. Goltz, J. Grossman, B. Issacs, F. Klasner, J. Kunna, A. Least, J. Loda, K. McMurry, S. Munson, J. O'Neill, L. W. Pratt, C. Radford, V. Robbins, N. Shema; H. Sin, B. Spurr, I. Stout, R. Sugihara, and G. Wright for assistance with field and/or laboratory work; B. Ashford for assistance with operating the bait bucket; D. Okita for piloting the helicopter; G. Arnold for statistical advice and analysis; E. Campbell, J. Eisemann, M. Fall, D. LaPointe, C. Natividad Bailey, L. W. Pratt, C. Swift, and T. Tunison for comments on the experimental use permit final report; S. Fortna, S. Hess, C. Swift, and E. Wilson Rankin for comments on this technical report and C. Bezar for editorial services. The research was conducted under Experimental Use Permit No. 73815-EUP-1 from the U. S. Environmental Protection Agency, with the approval of the U.S. National Park Service, U.S. Fish and Wildlife Service, Hawai'i Division of Forestry and Wildlife, and The Nature Conservancy, and in coordination with the Hawai'i Department of Agriculture. Funding was provided by the U.S. Geological Survey Invasive Species Program, USGS Natural Resources Protection Program, the U.S. Fish and Wildlife Service, the National Park Service Fee Demonstration Program and the New Zealand Foundation for Research, Science and Technology (CO9X0009). The authors would like to acknowledge additional support from the Pacific Cooperative Studies Unit, University of Hawai'i at Mānoa. Any use of trade, product, or firm names in this publication is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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