Technical Report HCSU-043

EFFICACY OF HAND-BROADCAST APPLICATION OF DIPHACINONE BAIT FOR RODENT CONTROL IN HAWAIIAN MONTANE FORESTS

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TABLE OF CONTENTS

LIST OF TABLES

List of Figures

ABSTRACT

Introduced black rats (*Rattus rattus*), Polynesian rats (*R. exulans*), and Norway rats (*R. norvegicus*) impact insular bird, plant, and invertebrate populations worldwide. We investigated the efficacy of hand-broadcast application of Ramik® Green containing 0.005% diphacinone for rodent control in paired 4-ha treatment and non-treatment plots in both wet and mesic forest in Hawai'i. Radio telemetry of black rats, the predominant species, indicated 100% mortality in both treatment plots within about one week of bait application. Live trapping and non-toxic census bait block monitoring two to four weeks after each of 12 repeat bait applications in the wet forest, and three repeat bait applications in the mesic forest, indicated rat abundance was reduced on average by 84–88%. However, reinvasion could have occurred within this time. Rat populations in the treatment plots usually recovered to pre-poison levels within two to five months. House mice (*Mus musculus*), Indian mongooses (*Herpestes auropunctatus*), and feral cats (*Felis catus*) also ate bait or other animals that had eaten bait. This study demonstrates the efficacy of ground-based broadcast toxicant baits for the control of rats in Hawaiian montane wet forests.

INTRODUCTION

Introduced small mammal predators have had devastating impacts on insular environments worldwide (Atkinson 1977, 1985; Buckle and Fenn 1992; Moors *et al.* 1992; Seto and Conant 1996; Towns and Broome 2003; Clout and Russell 2006; Hess and Jacobi 2011). In Hawai'i, four species of introduced rodents, the black rat (*Rattus rattus*), Polynesian rat (*R. exulans*), Norway rat (*R. norvegicus*), and house mouse (*Mus musculus*), together with the introduced feral cat (*Felis catus*) and Indian mongoose (*Herpestes auropunctatus*), are sympatric with native forest birds, plants, and invertebrates (Stone 1985, Tomich 1986, Sugihara 1997, Lindsey *et al.* 2009, Scheffler *et al.* 2012). The impacts of these predators on native communities are largely unquantified. However, rat predation of eggs, nestlings, and adult birds has been postulated as a leading cause of the accelerated decline and extirpation of endemic avian species and as a major factor limiting present populations of forest birds in Hawai'i (Atkinson 1977, 1985; Berger 1981; Scott *et al.* 1986; Lindsey *et al.* 2009). In addition, rats prey on native Hawaiian tree snails (*Achatinella* spp.; Hadfield *et al.* 1993) and insects (Sugihara 1997). Rats may also compete for food with the Hawaiian Crow (*Corvus hawaiiensis*), 'Ōma'o (*Myadestes obscurus*; Scott *et al.* 1986, Lindsey *et al.* 2009), and with some endemic insectivorous bird species such as the 'Akiapōlā'au (*Hemignathus munroi*) and Hawai'i Creeper (*Oreomystis mana*) that specialize on large conspicuous invertebrates (Stone and Scott 1985). Fruits and seeds of many endemic plant species are also susceptible to predation by rats (U.S. Fish and Wildlife Service 2002, Lindsey *et al.* 2009).

Only two methods (snap trapping and bait station application of diphacinone) are available for controlling rats in forested areas in Hawai'i (Nelson *et al.* 2002). Trapping and use of toxicants in bait stations can be effective short-term methods of control in small or limited areas. However, both are labor-intensive and impractical for controlling pests over large areas (Moors *et al.* 1992, Tobin 1994, Nelson *et al.* 2002, Towns and Broome 2003, Hess *et al.* 2009).

Hand- and aerial-broadcast application of baits containing anticoagulant rodenticides have been used successfully to control introduced rodents for species conservation and ecosystem restoration in New Zealand (Innes *et al.* 1995, Towns and Broome 2003, Clout and Russell

2006, Hess *et al.* 2009). The success of these pest-control efforts prompted the formation of a multi-agency rodenticide working group to seek regulatory approval for the use of similar techniques in Hawai‘i. Diphacinone, a first-generation anticoagulant, was the rodenticide selected to pursue for registration because of its demonstrated effectiveness against rats, low risk to non-target species, and short persistence in the environment (Swift 1998, Nelson *et al.* 2002, Hess *et al.* 2009).

Rats are highly sensitive to diphacinone, though multiple feedings are generally required to cause mortality (Swift 1998). In a series of laboratory bioassays with rats caught in a forest near Hilo, Hawai‘i, Swift determined exposure times and doses of 7 days and 37.5 g of 0.005% diphacinone bait for *R. rattus* and 6 days and 30.0 g for *R. exulans* caused 80% and 90% mortality, respectively, under laboratory conditions. As with all anticoagulant rodenticides, consumption does not result in immediate poisoning symptoms, avoiding the development of bait shyness that arises when individuals associate the symptoms with the food item just consumed (Swift 1998).

We evaluated the efficacy of hand-broadcast application of cereal-based pellet baits containing 0.005% (50 ppm) diphacinone for rodent control in Hawai‘i, to generate supporting data needed for state and federal regulatory approval of a pesticide label permitting application of a commercial rodenticide bait product (Ramik® Green) using this method. The research was conducted under two State of Hawai‘i Experimental Use Permits (EUP-99-01 and EUP-99-02) with the approval of the U.S. National Park Service, U.S. Fish and Wildlife Service, State of Hawai‘i Division of Forestry and Wildlife, and The Nature Conservancy of Hawai‘i in coordination with the Hawai‘i Department of Agriculture.

METHODS

Study Design

The Experimental Use Permits issued by the State of Hawai‘i Department of Agriculture’s Pesticide Branch allowed trials to be done in both wet forest and mesic forest in Hawai‘i Volcanoes National Park (HAVO). However, the permits limited the treatment area in each forest type to 4 ha with no replication allowed. Consequently, we had only one treatment plot (200 × 200 m) in each forest type. Each was paired with a same-sized non-treatment plot. A rodent monitoring grid was established in each plot, consisting of 17 transect lines, 12.5 m apart. Each transect line was flagged with markers at 12.5-m intervals. Although not true replicates (because they were in different forest types), the two treatment plots allowed us to compare the generality of treatment.

The paired wet forest plots were in the southwest corner of the Koa Management Unit in ‘Ōla‘a Forest on the lower slope of Mauna Loa, HAVO, at approximately 1200 m elevation. This 800-ha unit was fenced in 1990 and has been free of feral pigs (*Sus scrofa*) since 1994. The forest was composed of an open canopy of scattered, large ‘ōhi‘a trees (*Metrosideros polymorpha*) with a diverse middle story of mixed native trees including *Cheirodendron*, *Perotettia*, and *Ilex* and a dense understory of tree ferns (*Cibotium* spp.). Ground cover consisted primarily of native ferns, shrubs, and sedges, but a few alien plants were also common, particularly yellow Himalayan raspberry (*Rubus ellipticus*) and banana poka (*Passiflora tarminiana*). Average annual rainfall is approximately 2500 mm. The treatment plot (HAVO transect 16) was separated from the non-treatment plot (HAVO transect 18) by 450 m. This distance was considered sufficient to prevent most rats moving between plots (see discussion below).

The paired mesic forest plots (Kīpuka Kī and Kīpuka Puaulu) were on deep ash soil surrounded by lava of the late prehistoric Keamoku flows on the lower slope of Mauna Loa, HAVO, at 1200–1360 m elevation (Mueller-Dombois and Lamoureux 1967), approximately 8 km west of the wet forest study area. The vegetation in the central part of both kīpuka was tall forest comprised of koa, ʻōhiʻa, and soapberry (*Sapindus saponaria*). Ground cover was dominated by native ferns and herbs where the forest canopy was dense, but blackberry (*Rubus argutus*) and alien grasses, such as meadow ricegrass (*Ehrharta stipoides*) and *Paspalum* spp., were common in some areas, and patches of open grassland occurred where trees were only scattered. Kīpuka Kī also contained some Jerusalem cherry (*Solanum pseudocapsicum*). Kīpuka Puaulu was fenced against cattle in the 1930s and has been free of feral pigs since the mid-1960s. Kīpuka Kī was fenced against cattle in the late 1940s and has been free of feral pigs since the mid-1980s. Average annual rainfall is 1200 mm. The treatment plot (Kīpuka Kī) was separated from the non-treatment plot (Kīpuka Puaulu) by about 1.5 km.

Bait Application

The test bait was a cereal-based, fish-flavored, green-colored pellet formulation of Ramik® Green (HACCO Inc., Madison, WI), nominally weighing 6 g and containing 0.005% (50 ppm) diphacinone. Two lots of baits were used: Lot No. 125218 manufactured on 4 May 1999 (used in trials from October 1999 to October 2000), and Lot No. 144548 manufactured on 16 September 2000 (used in trials from November 2000 to December 2001). The diphacinone concentration of both lots of bait, measured using high-performance liquid chromatography (HPLC), was 51 ppm at the time of manufacture (Mary Ann Douglas, HACCO Inc., personal communication). This is within the limits certified in the Code of Federal Regulations (45–55 ppm). The diphacinone concentration (mean ± SE) in a random sample of Lot No. 125218 was 46.4 ± 0.6 ppm, still within the certified limits, 12 months after manufacture (Genesis Laboratories Inc., Wellington, CO). The diphacinone concentration in random samples of Lot No. 144548 was 40.3 ± 0.9 ppm 16 months after manufacture, 39.4 ± 0.7 ppm 19 months after manufacture (both measured by Genesis Laboratories Inc.), and 44 ppm 20 months after manufacture (measured by HACCO Inc.).

Bait was applied by trained personnel who walked along each transect line and, every 2.5 m, threw single baits about 1, 3, and 5 m to each side. The rate of bait application was 22.4 kg/ha. Half of the bait (11.2 kg/ha) was applied on day 1 and the other half applied four to six days later. This ensured that baits were available to rats over the recommended time period of 10–15 days (Dunlevy *et al.* 2000). Bait was first hand broadcast in the wet forest treatment plot on 7 and 12 October 1999 (when five months old), and in the mesic forest treatment plot on 27 January and 1 February 2000 (when nine months old). Eleven further hand-broadcast series (composing two applications of bait, four to six days apart) were made at two- to four-month intervals until December 2001 in the wet forest treatment plot, and two further hand-broadcast series were made at three- to five-month intervals in the mesic forest treatment plot. The exact timing of application was determined by crew availability and weather conditions. The baits were applied only when the ground was reasonably dry and predictions were for favorable weather over the next five days.

The average daily rainfall in the wet forest treatment plot, measured with a rain gauge, was less than 5 mm in the two weeks after all bait applications except in October 1999, December 1999, and November 2000. In October 1999, an average of 2.4 mm of rain fell during the first 13 days, but then 51 mm fell on day 14. In December 1999, 174 mm of rain fell on day 2 and 280 mm on day 3. In November 2000, 49 mm of rain fell on day 2, 17 mm on day 4, and

49 mm on day 5. Maximum daily temperatures during the two weeks after bait application ranged from 17.5 to 22.9°C, and minimum temperatures ranged from 7.4 to 14.3°C.

Little rain fell in the mesic forest treatment plot in the first two weeks following the first two bait application series in January and July 2000 (average 0.1 and 1.6 mm daily, respectively). However, 24 mm of rain fell on day 2, and more than 50 mm fell on day 7 and again on day 8, after the third bait application series in October 2000 (average 6.1 mm daily). Maximum daily temperatures ranged from 23.1 to 26.4°C, and minimum temperatures ranged from 6.3 to 12.7°C.

Impact on Rat Abundance

Radio telemetry

Radio transmitters (weighing 4.2 g, Model PD-2C, Holohil Systems, Ontario, Canada) were fitted to six black rats (all that could be caught in the time available) in the treatment plot and 13 black rats in the non-treatment plot in the wet forest, one week before the first bait application in October 1999. However, one rat in the treatment plot and two rats in the non-treatment plot slipped their radio-transmitter collars before bait application. Radio transmitters were also fitted to 17 black rats in the treatment plot and 15 black rats in the non-treatment plot in the mesic forest, one week before the first bait application there in January 2000. However, one transmitter in the non-treatment plot stopped functioning and another slipped off before bait application. Radio signals from the radio-collared rats were monitored using portable receivers (Telonics, Mesa, AZ, model TR-4) and hand-held two-element directional antennas (Telonics RA-14). Each rat was monitored nightly for three consecutive nights immediately before bait application and nightly for up to two weeks after bait application to determine whether it was alive. A fluctuating, variable-strength radio signal indicated that the rat was active and alive, whereas a constant, steady radio signal indicated that the rat was not moving and possibly dead. Each rat not moving during a nightly monitoring session was tracked to its location the next day in an attempt to determine its fate. The carcasses of rats found dead were collected for necropsy and analysis of diphacinone residues using HPLC at Genesis Laboratories Inc. (Wellington, CO) or Landcare Research (Lincoln, New Zealand).

The percentage reduction in the proportion of radio-collared rats surviving in the treatment plot, adjusted for any reduction in survival in the non-treatment plot, was calculated from the formula:

$$\%\,reduction = 100 \times ((E - O) / (E)) \qquad (1)$$

where E = expected number in treatment plot pre-treatment × (number in non-treatment plot post-treatment / number in non-treatment plot pre-treatment), and O = observed number in treatment plot post-treatment.

The statistical significance of bait application on the survival of radio-collared rats in the mesic forest was assessed by a 2 × 2 chi-square analysis of the number of radio-collared rats alive vs. dead, pre- and post-treatment, in the treatment and non-treatment plots. It was not possible to analyze for the effect of bait application on the survival of radio-collared rats in the wet forest because too few rats were radio collared in the treatment plot.

Live trapping

A total of 81 Haguruma® wire cage-traps were placed at 25-m intervals on transect lines spaced 25 m apart (i.e., every second transect line) within each study plot two weeks before the first trapping and left closed to allow rats time to become accustomed to the traps. The traps then remained at the trap locations throughout the study period with broken traps replaced when necessary. Two weeks before toxic baits were hand broadcast within the treatment plots, trap locations were pre-baited with shredded coconut. Three days later, the traps were opened and baited with coconut chunks. The traps were checked daily for four consecutive days (maximum 324 trap-nights), and all captured rats that did not escape while being handled were identified to species, sex, and age class (juvenile or adult), weighed, ear-tagged, and then released. The traps were operated again, as above, two to four weeks after each bait application series to determine the efficacy of the baiting. Rat capture rates 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, in each forest type was calculated in the same way as for the radio-telemetry data (formula 1), but exchanging “number” for “capture rate.” The statistical significance of bait application on rat capture rates was determined for each series of bait applications using a generalized linear model (GLM; S-Plus for Windows, 2001, Insightful Corporation, Seattle, WA), adjusting for the number of trap-nights by using log (trap-nights) as an offset term in the model. The ratio of the variance to the mean (a measure of dispersion) was estimated separately for the wet forest and mesic forest, from the “plot by time” interaction in a model of the pre-treatment capture rates in the treatment and non-treatment plots for the 12 bait application series in the wet forest and three bait application series in the mesic forest. These values were used to scale the residual deviances of the generalized linear model before assessing their significance against a chi-square distribution (McCullagh and Nelder 1989).

Non-toxic census blocks

Seventy-two non-toxic, cereal and wax-based CENSUS® Bait Blocks (gnaw blocks or chew blocks; hereafter, census blocks; Zeneca Professional Products, Wilmington, DE) were placed at 25-m intervals on the same transect lines as live traps within each study plot, but halfway between the live trap locations, one to three weeks before and after each bait application series. They were attached to the ground using a 1-m wire flag inserted through the center of each census block. The census blocks were examined daily for two consecutive days (maximum 144 bait-nights) for diagnostic signs of feeding by rats or other animals (e.g., mice, birds, and invertebrates). The percentage reduction in the number of census blocks gnawed or supposedly removed by rats in the treatment plot relative to the non-treatment plot and the effectiveness of each bait application series were calculated in the same way as for the live-trapping data.

No serious attempt was made to monitor populations of other pest species such as house mice, mongooses, or feral cats. However, mice were caught in the live traps used for monitoring rats, and consequently mouse capture rates were calculated in the same way as for rats. Mice also left distinctive gnaw marks on the census blocks used for monitoring rats, and the percentage of census blocks gnawed by mice was calculated in the same way as for rats. Signs of mice, mongooses, and feral cats feeding on baits (e.g., green-dyed feces) were recorded, and carcasses of dead animals were collected for necropsy and analysis of diphacinone residues as above.

RESULTS

Impact on Rat Abundance in Wet Forest

Wet forest radio telemetry

All five radio-collared rats in the treatment plot were found dead 5–7 days (average 5.4 days) after the initial bait application in early October 1999. None of the 11 radio-collared rats in the non-treatment plot were found dead, but two radio transmitters were found on the ground. One was from a rat that appeared to have been eaten by a mammalian predator, because rat fur and bone fragments were located with the transmitter. It was not possible to determine whether the rat had been eaten before or after it died. The second transmitter was recovered without a rat, and thus the fate of the rat could not be determined. Post-treatment survival of the radio-collared rats in the treatment plot (0 of 5 rats) and non-treatment plot (9 of 11 rats) could not be compared statistically because the sample sizes were too small.

All five radio-collared rats recovered in the treatment plot had internal hemorrhaging (under skin, around heart, or in lungs, liver, bladder, genitals, thoracic cavity, or abdominal cavity), typical of anticoagulant poisoning. Four rats also had external hemorrhaging (from mouth, ear, or genital region) and green bait in their stomachs or green fecal pellets in their intestines. The average diphacinone concentration in the livers of the five rats was 3.7 ppm (range 0.5–8.1 ppm).

Wet forest live trapping

One week before the initial bait application in early October 1999, 14 black rats were captured in the treatment plot (4.48 rats per 100 corrected trap-nights) and 17 black rats in the non-treatment plot (5.58 rats per 100 corrected trap-nights; Table 1). Two weeks after bait application, no rats were caught in the treatment plot, but 25 rats (22 black rats and 3 Norway rats) were captured in the non-treatment plot (8.47 rats per 100 corrected trap-nights). The number of rats captured in the treatment plot two weeks after bait application was lower than expected from the number captured in the non-treatment plot (Table 1). However, rat capture rates in the treatment plot exceeded pre-treatment levels by the end of November 1999, nearly eight weeks after bait application (Figure 1).

Repeat bait applications at two- to four-month intervals over two years resulted in rat capture rates in the treatment plot being reduced to zero or near zero 2–4 weeks after each bait application series, except in June and August 2000 (Table 1 and Figure 1). Reductions of less than 100% were not statistically significant at the 95% level of probability (Table 1). Capture rates in the treatment plot increased to near or above pre-treatment levels within 8–12 weeks after each bait application series (Table 1 and Figure 1). Capture rates in the non-treatment plot also fluctuated over time, but not in response to bait application (Figure 1).

Of the rats captured, 74% were black rats, 17.4% were Polynesian rats, and 8.6% were Norway rats (n = 689). Most captured rats were adult males (35.8%), followed by adult females (22.4%), juvenile males (19.8%), juvenile females (12.0%), and unknown (9.9%; n = 656).

None of the 28 rats caught in the treatment plot two to four weeks after bait application (all applications combined) had been ear-tagged in the treatment plot before bait application (i.e., they were not recaptures), whereas 22% of the 199 rats captured in the non-treatment plot

Table 1. Rat live captures per 100 corrected trap-nights in non-treatment and treatment plots one to two weeks before and two to four weeks after repeat hand-broadcast applications of Ramik® Green bait in wet forest, Hawai‘i Volcanoes National Park, Hawai‘i.

Date start bait	Non-treatment plot			Treatment plot			% reduction in	GLM	*P* value
application	Before	After	% change	Before	After	% change	treatment plot	χ^2 value	(1 df)
7 Oct 1999	5.58	8.47	+51.8	4.48	0	−100	100	6.64	0.010
8 Dec 1999	4.07	4.29	+5.3	7.10	0	−100	100	6.49	0.011
8 Feb 2000	4.20	6.59	+56.9	5.58	0	−100	100	7.61	0.006
12 Apr 2000	3.69	7.36	+99.3	7.99	0	−100	100	10.86	0.001
14 Jun 2000	9.81	8.06	−17.8	7.83	4.59	−41.4	29	0.14	0.706
31 Aug 2000	5.41	9.93	+83.7	4.62	2.55	−44.9	70	1.65	0.199
9 Nov 2000	12.82	4.20	−67.2	18.61	0	−100	100	6.55	0.010
18 Jan 2001	3.93	2.28	−41.9	10.40	0.32	−97.0	95	3.42	0.064
19 Mar 2001	5.04	3.45	−31.6	3.97	0.63	−84.2	77	1.07	0.301
24 May 2001	6.03	1.90	−68.5	13.41	0.74	−94.5	83	1.37	0.242
23 Aug 2001	4.18	5.01	+19.7	3.40	0	−100	100	3.87	0.049
17 Dec 2001	12.50	6.95	−44.4	7.45	0.64	−91.5	85	2.45	0.117

GLM = generalized linear model

two to four weeks after bait application were recaptures (χ^2 = 13.406, df = 1, P < 0.001). However, one rat (an adult female black rat) captured in the treatment plot nine weeks after the August 2000 bait application had been ear-tagged in the treatment plot seven weeks before bait application and so had either survived the bait application or been away from the treatment plot while bait was present. Four rats captured in the treatment plot 3, 7, 8, and 10 weeks, respectively, after bait application had been ear-tagged in the non-treatment plot at least 500 m away (the plots were 450 m apart and traps were at least 25 m inside plot edges). They may or may not have been present in the treatment plot at the time of bait application.

Wet forest non-toxic census bait blocks

Before the initial bait application in early October 1999, rats interfered with 29.6% of census blocks in the treatment plot (26.8% of blocks gnawed by rats plus 2.8% of blocks missing, presumably removed by rats) and 36.1% in the non-treatment plot (31.9% gnawed by rats plus 4.2% missing; Table 2 and Figure 2). Three weeks after the initial bait application, rat interference had declined

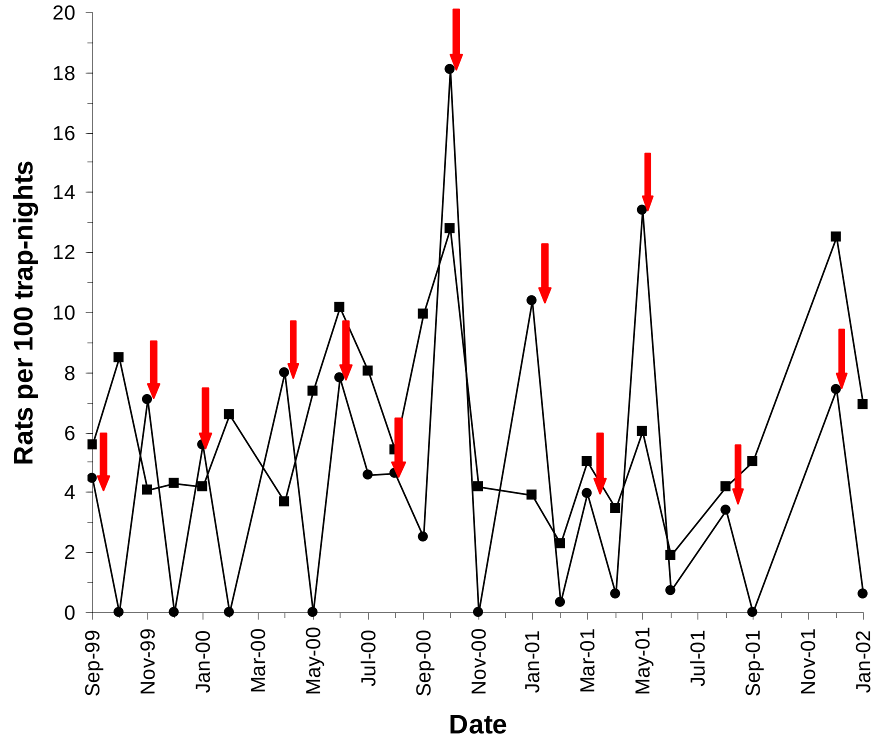

Figure 1. Live captures of rats (per 100 corrected trap-nights) in wet forest treatment (●) and non-treatment (■) plots, Hawai‘i Volcanoes National Park, Hawai‘i. Arrows indicate date of bait applications.

to 1.4% in the treatment plot (no blocks missing), but had increased to 87.5% in the non-treatment plot (62.5% gnawed by rats, 25.0% missing). The reduction in rat interference to census blocks in the treatment plot relative to the non-treatment plot was, therefore, 98.0% (χ^2 = 378.45, df = 1, P < 0.001 on raw data). However, by late November/early December 1999, seven weeks after the initial bait application, rat interference to census blocks had increased to 12.5% in the treatment plot (no blocks missing) and 95.8% in the non-treatment plot (35.2% gnawed by rats, 60.6% missing; Table 2 and Figure 2).

Repeated bait applications at two- to four-month intervals over two years reduced rat interference to census blocks in the treatment plot to zero or near zero each time, except in May 2001 when it was reduced only to 12.5% (Table 2 and Figure 2). At the same time, rat interference (gnawed and missing blocks) in the non-treatment plot fluctuated from about 93 to 100%. Most bait applications caused a significant reduction in rat interference to census blocks in the treatment plot compared with the non-treatment plot (Table 2).

Impact on Rat Abundance in Mesic Forest

Mesic forest radio telemetry

All 17 radio-collared rats in the treatment plot were considered to be dead 4–6 days (average 4.9 days) after the initial bait application in January 2000. However, only six could be recovered

Table 2. Percentage of census bait blocks gnawed or removed by rats in non-treatment and treatment plots one to two weeks before and two to three weeks after repeat hand-broadcast applications of Ramik® Green bait in wet forest, Hawai‘i Volcanoes National Park, Hawai‘i.

Date start bait application	Non-treatment plot			Treatment plot			% reduction in treatment plot	GLM χ^2 value	*P* value (1 df)
	Before	After	% change	Before	After	% change			
7 Oct 1999	36.1	87.5	+142.4	29.6	1.4	−95.3	98	378.45	<0.001
8 Dec 1999	95.8	98.6	+2.9	12.5	5.6	−55.2	57	15.75	<0.001
8 Feb 2000	97.2	100.0	+2.9	2.7	0	−100	100	19.43	<0.001
12 Apr 2000	100.0	97.2	−2.8	15.3	1.4	−90.8	91	4.02	0.045
14 Jun 2000	100.0	98.6	−1.4	23.6	5.6	−76.3	76	3.36	0.067
31 Aug 2000	93.1	100.0	+7.4	72.2	1.4	−98.1	98	204.67	<0.001
9 Nov 2000	98.6	100.0	+1.4	52.8	4.2	−92.0	92	23.55	<0.001
18 Jan 2001	100.0	100.0	0	–	0	−100	100	–	–
19 Mar 2001	100.0	100.0	0	9.9	0	−100	100	18.07	<0.001
24 May 2001	100.0	100.0	0	22.1	12.5	−43.4	43	0.91	0.340
23 Aug 2001	100.0	100.0	0	54.2	5.6	−89.7	90	13.71	<0.001
17 Dec 2001	93.0	100.0	+7.5	75.0	4.2	−94.4	95	183.24	<0.001

GLM = generalized linear model

and confirmed dead. The other 11 rats were high in the canopy or under immovable rocks and could not be recovered, but because they remained stationary during daily monitoring for more than a week they were presumed dead. Of the 13 radio-collared rats in the non-treatment plot, one stopped moving and was presumed dead four days after application of bait in the treatment plot. This rat was not recovered and was recorded as a natural mortality. Post-treatment survival of the radio-collared rats (0 of 17 rats) in the treatment plot was significantly lower than in the non-treatment plot (12 of 13 rats; $\chi^2 = 26.154$, df = 1, $P < 0.001$).

Five of the six radio-collared rats recovered in the treatment plot were necropsied and all had internal hemorrhaging (under skin, around heart, or in lungs, liver, bladder, genitals, thoracic cavity, or abdominal cavity) typical of anticoagulant poisoning. Three also had external hemorrhaging (from nose and genital region) and green bait in their stomachs or green fecal pellets in their intestines. The average diphacinone concentration in the livers of the five rats was 3.4 ppm (range 1.8–5.0 ppm).

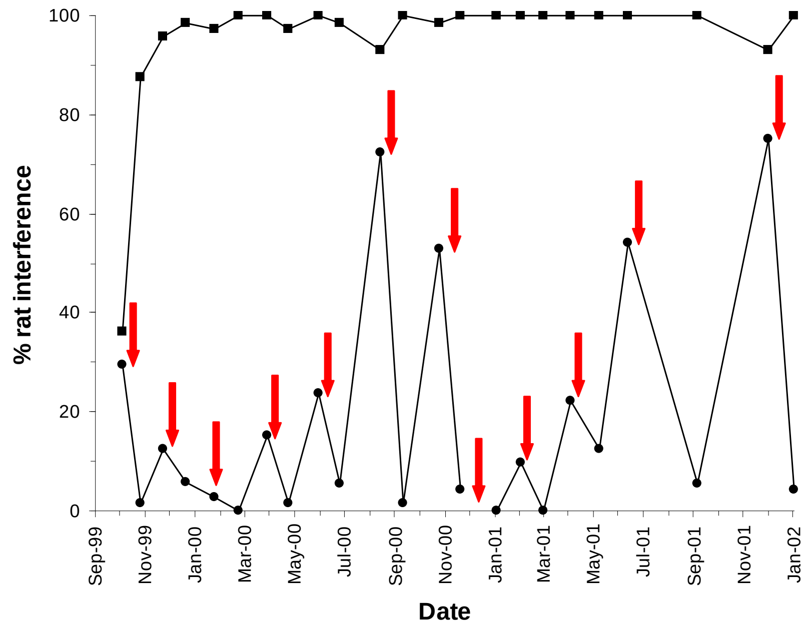

Figure 2. Percentage of non-toxic census bait blocks interfered with by rats in wet forest treatment (●) and non-treatment (■) plots, Hawai‘i Volcanoes National Park, Hawai‘i. Arrows indicate date of bait applications.

Mesic forest live trapping

One week before the initial bait application in January 2000, 29 rats (28 black rats and 1 Polynesian rat) were captured in the treatment plot (10.86 rats per 100 corrected trap-nights), and 32 rats (all black rats) were captured in the non-treatment plot (12.48 rats per 100 corrected trap-nights; Table 3 and Figure 3). Two weeks after bait application, zero rats were captured in the treatment plot, and 47 rats (46 black rats and 1 Polynesian rat) were captured in the non-treatment plot (17.87 rats per 100 corrected trap-nights). The decline in rat capture rate in the treatment plot relative to the non-treatment plot (100%) was significant (χ^2 = 24.02, df = 1, P < 0.001). Seven weeks after bait application, 1 rat (a Polynesian rat) was caught in the treatment plot (0.32 rats per 100 corrected trap-nights), and 27 rats (all black rats) were caught in the non-treatment plot (9.75 rats per 100 corrected trap-nights). It was not until 21 weeks after the initial bait application (i.e., in June 2000) that the rat capture rate in the treatment plot increased to more than 50% of the pre-treatment capture rate (Figure 3).

Three weeks after the second bait application series in July 2000, rat captures in the treatment plot declined from 17 black rats (6.34 rats per 100 corrected trap-nights) to 1 black rat (0.34 rats per 100 corrected trap-nights; Figure 3). However, rat captures also declined in the non-treatment plot, from 29 black rats (9.62 rats per 100 corrected trap-nights) to 9 black rats (2.98 rats per 100 corrected trap-nights). The decline in the treatment plot relative to the non-treatment plot (83%) was not statistically significant (χ^2 = 2.05, df = 1, P = 0.152).

Three weeks after the third bait application series in October 2000, rat captures declined from 19 rats (all black rats) to 1 rat (a black rat) in the treatment plot and from 25 rats (23 black

Table 3. Rat live captures per 100 corrected trap-nights in non-treatment and treatment plots one week before and two to three weeks after repeat hand-broadcast applications of Ramik® Green bait in mesic forest, Hawai‘i Volcanoes National Park.

Date start bait application	Non-treatment plot			Treatment plot			% Reduction in treatment plot	GLM χ^2 value	*P* value (1 df)
	Before	After	% change	Before	After	% change			
27 Jan 2000	12.48	17.87	+43.2	10.86	0	−100	100	24.02	<0.001
5 Jul 2000	9.62	2.98	−69.9	6.34	0.34	−94.6	83	2.05	0.152
25 Oct 2000	8.67	7.39	−14.8	6.83	0.32	−95.3	95	7.38	0.007

GLM = generalized linear model

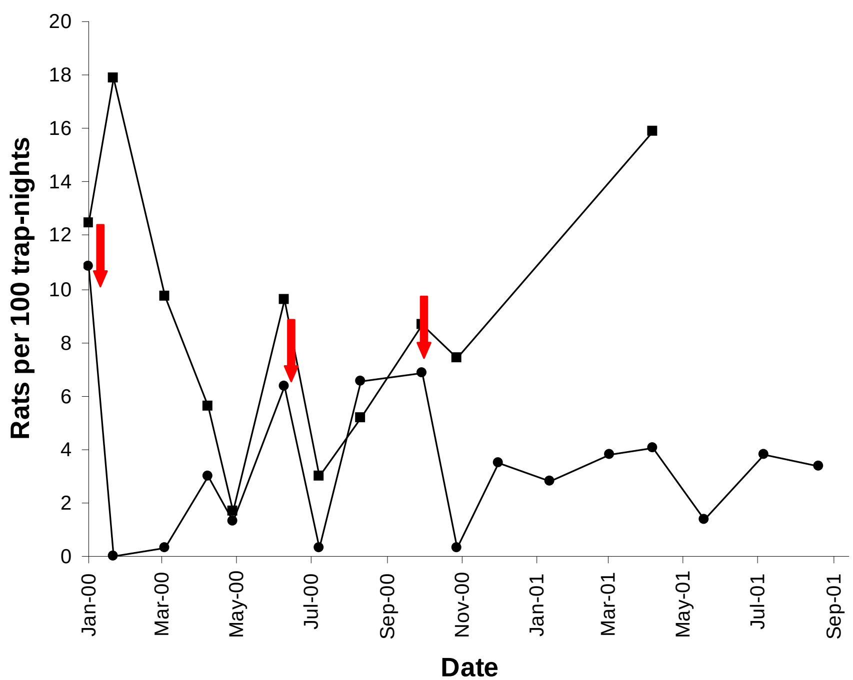

Figure 3. Live captures of rats (per 100 corrected trap-nights) in mesic forest treatment (●) and non-treatment (■) plots, Hawai‘i Volcanoes National Park, Hawai‘i. Arrows indicate date of bait applications.

rats, 1 Polynesian rat, and 1 Norway rat) to 21 rats (19 black rats and 2 Polynesian rats) in the non-treatment plot (Figure 3). The decline in the treatment plot relative to the non-treatment plot (95%) was statistically significant (χ^2 = 7.38, df = 1, P = 0.007). The rat capture rate in the treatment plot did not increase above 4 rats per 100 corrected trap-nights during the year after the third bait application series, less than half the initial capture rate, and further bait applications were not made (Figure 3).

Of all the rats captured, 97% were black rats, 1.7% were Polynesian rats, and 1.3% were Norway rats (n = 230). Most captured rats were adult males (36.6%), followed by adult females (30.8%), juvenile males (13.9%), juvenile females (12.0%), and unknown (6.7%; n = 208).

Neither of the two rats captured in the treatment plot two to three weeks after bait application (all applications combined) had been recaptured (i.e., they had not been ear-tagged before treatment). However, on average, 22% of the 77 rats captured in the non-treatment plot had been recaptured.

Mesic forest non-toxic census blocks
Before the initial bait application in January 2000, rats interfered with 5.6% of the census blocks in the treatment plot (no blocks missing) and 56.9% in the non-treatment plot (41.7% gnawed by rats plus 15.3% of blocks missing, presumably taken by rats; Table 4 and Figure 4). Three weeks after the initial bait application, rat interference had declined to 0% in the treatment plot (100% reduction), but increased to 62.5% in the non-treatment plot (37.5% gnawed by rats, 25.0% missing). The reduction in rat interference to census blocks in the treatment plot relative to the non-treatment plot was statistically significant (χ^2 = 36.56, df = 1, P < 0.001).

Repeat bait applications reduced the percentage of census blocks in the treatment plot interfered with by rats in July 2000 from 2.8% to 1.4% (no blocks missing before or after treatment) and in October 2000 from 34.7% (20.8% gnawed by rats, 13.9% missing) to 12.5% (4.2% gnawed by rats, 8.3% missing; Table 4 and Figure 4). In July 2000, the percentage of census blocks in the non-treatment plot interfered with by rats decreased from 73.6% (27.8% gnawed by rats, 45.8% missing) to 66.7% (23.6% gnawed by rats, 43.1% missing) and increased from 77.8% (11.1% gnawed by rats, 66.7% missing) to 88.9% (22.2% gnawed by rats, 66.7% missing) in October 2000. The reduction in rat interference to census blocks in the treatment plot relative to the non-treatment plot was 44.8% in July 2000 (χ^2 = 0.52, df = 1, P = 0.469) and 68.5% in October 2000 (χ^2 = 71.1, df = 1, P < 0.001).

Impact on Other Pest Species
Too few mice were caught in the wet forest treatment plot (average <1 per 100 corrected trap-nights) and too few census blocks were interfered with by mice (average 0.8%) to warrant rigorous examination of the data. However, overall, there was a reduction in mouse capture rates in the treatment plot relative to the non-treatment plot of 75.7% (average of all applications combined) and a reduction in mouse interference to census blocks in the treatment plot relative to the non-treatment plot of 69.2% (average of all applications combined). Two mice found dead in the wet forest treatment plot three weeks after bait application contained 2.39 and 1.75 ppm diphacinone, respectively, in their livers.

The mouse capture rate in the mesic forest treatment plot was reduced by 100% two weeks after the first bait application series in January 2000 and by 95% three weeks after the second

Table 4. Percentage of census bait blocks gnawed or removed by rats in non-treatment and treatment plots, one to two weeks before and two to three weeks after repeat hand-broadcast applications of Ramik® Green bait in the mesic forest, Hawai'i Volcanoes National Park.

Date start bait application	Non-treatment plot			Treatment plot			% Reduction in treatment plot	GLM χ^2 value	*P* value (1 df)
	Before	After	% change	Before	After	% change			
27 Jan 2000	56.9	62.5	+9.8	5.6	0	−100	100	36.56	<0.001
5 Jul 2000	73.6	66.7	−9.4	2.8	1.4	−50.0	45	0.52	0.469
25 Oct 2000	77.8	88.9	+14.3	34.7	12.5	−64.0	69	71.10	<0.001

GLM = generalized linear model

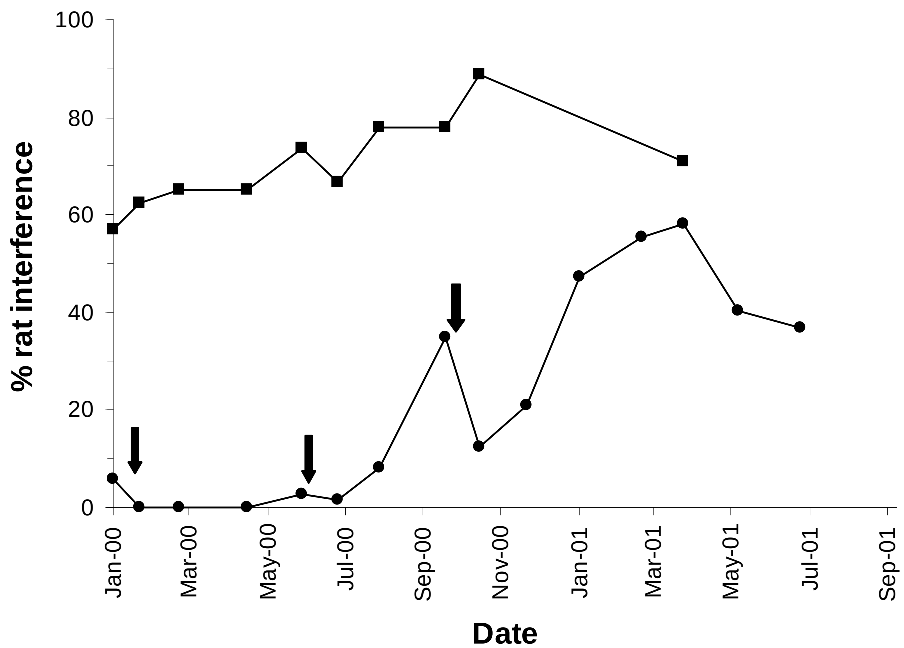

Figure 4. Percentage of non-toxic census bait blocks interfered with by rats in mesic forest treatment (●) and non-treatment (■) plots, Hawai'i Volcanoes National Park, Hawai'i. Arrows indicate date of bait applications.

and third bait application series in July 2000 (χ^2 = 7.87, df = 1, P = 0.005) and October 2000 (χ^2 = 17.1, df = 1, P < 0.001). Mouse gnawing on census blocks in the mesic forest treatment plot was reduced by 100% (from 9.7% to 0%) after the first bait application series in January 2000. However, there was no mouse gnawing on census blocks in the treatment plot before or after either of the other two bait application series. Forty-five percent (5/11) of the mice found dead in traps (i.e., captured alive) two to three weeks after bait application in the mesic forest contained diphacinone in their livers (average 0.88 ppm, range 0.03–3.2 ppm).

A juvenile male mongoose was found dead in the mesic forest treatment plot on 19 July 2000, two weeks after bait application. A necropsy revealed hemorrhaging typical of diphacinone poisoning. The liver contained 1.35 ppm diphacinone. Green-dyed mongoose feces were found in the mesic forest treatment plot on 11 February 2000, two weeks after bait application.

No feral cats were found dead. However, green-dyed feces of feral cats were found in the wet forest treatment plot on 29 December 1999 (21 days after bait application) and 11 and 22 February 2000 (3 and 14 days after bait application).

Discussion

The results from this study demonstrate that Ramik Green bait containing 0.005% (50 ppm) diphacinone, hand-broadcast at 22.4 kg/ha, in two applications of 11.2 kg/ha, four to six days apart, is effective in reducing populations of rats (predominantly black rats) in both wet and mesic forest habitat in Hawai‘i. A 100% reduction in the resident black rat population one week after the initial bait application series in both forest types was measured by radio telemetry. Live trapping and non-toxic census blocks indicated a 98–100% reduction in rat numbers two to four weeks after bait application. However, rat presence detected within this time could be attributed to reinvasion rather than surviving individuals, so these latter two methods may have underestimated efficacy. The synchronous death of rats following the application of toxic baits, together with the presence of bait in their stomachs, internal and external hemorrhaging, and diphacinone residues in the liver, indicate that the rats most likely died of diphacinone poisoning. Rat abundance usually recovered to pre-poison levels within two to five months of bait application, presumably by reinvasion of rats from surrounding areas.

Subsequent, repeat, hand-broadcast applications of bait in the two forest types were highly effective in reducing rat abundance on average by 88% (range 29–100%) as measured by live trapping and 84% (range 43–100%) as measured by census blocks, two to four weeks after bait application. There were some discrepancies between these two methods of monitoring for efficacy of reducing rat abundance. For example, in the wet forest in June 2000, the efficacy was 29% as measured by live trapping and 76% as measured by census blocks, and in August 2000 it was 70% as measured by live trapping and 98% as measured by census blocks. It is difficult to know which of the two methods was more accurate, but the estimate of efficacy in June 2000 was low by both methods. As noted above, reinvasion could have occurred in the two to four weeks after bait application, in which case both live trapping and census blocks have underestimated efficacy.

Different methods of monitoring the efficacy of toxic bait applications had different strengths and weaknesses. Radio telemetry was best because it enabled both the location and fate of known individuals to be determined. Radio-collared rats that lost their radio transmitters, died, or moved out of the study plots could be excluded from the data analysis. However, radio telemetry is costly and time-consuming. Consequently, it was used to monitor only the first bait application series in each treatment plot, and sample sizes were necessarily small, which limited the power of detecting survivors.

Live trapping, with ear-tagging, was also useful, but it was not possible to determine whether rats captured without ear-tags were present before bait application and survived (by not encountering baits, encountering baits but not eating them, or eating insufficient bait), or whether they had only moved into the plots after bait had disappeared or disintegrated. The small size of the study plots (200 × 200 m), the ability of rats to move over distances of several hundred meters, and the length of time between bait application and live trapping (up to four weeks), mean that rats from surrounding areas could have moved into the plots in the interim, masking the true efficacy of the treatment. None of the rats ear-tagged in the treatment plots before treatment in either forest type were recaptured in the first two to four weeks after treatment, supporting the interpretation that those rats that were captured then were immigrants into the treatment plots rather than survivors of the treatment. One ear-tagged rat was recaptured in the treatment plot in the wet forest nine weeks after treatment, and this may have been a survivor of the treatment, but equally may have moved out of the treatment plot before treatment, then moved back in again after treatment. This latter interpretation is plausible because four other rats moved at least 500 m among the treatment and non-treatment plots in three to ten weeks. The use of radio telemetry could have resolved this issue.

Gnawing on non-toxic census blocks was the most difficult method to interpret. In addition to the delay between bait application and post-treatment monitoring, allowing immigration or reinvasion of rats to mask the true efficacy, as in live trapping, other difficulties included (a) deciding which species (rats, mice, birds, or invertebrates) had gnawed the census blocks, (b) interpreting the meaning of 100% of the census blocks being gnawed or removed, and (c) deciding what to do about missing census blocks. For example, it was not always easy to determine which species had gnawed the census blocks, because gnawing by one species may have been masked by subsequent gnawing by another species. This may have resulted in an underestimate of the abundance of some species. When 100% of the census blocks have been gnawed or removed by rats, the maximum size of the rat population cannot be estimated. Even when interference approached 100%, the rat population was likely to be underestimated. Limiting the period of monitoring to one day might help reduce the level of interference for the first monitoring session, but it does not help for subsequent monitoring sessions, because rats learned where the census blocks were located and returned to them rather than interfering with them at random. The technique also had limitations when census blocks went missing, because it was not possible to determine which species was responsible for them going missing. Missing census blocks could have been deleted from the calculations, but this would have exaggerated the percentage interference to the remaining blocks by less common species. For example, if 50% of the blocks are missing, and rats gnawed 90% and mice 10% of the remaining blocks, and rats were responsible for removing the missing blocks, then the rat population will have been underestimated and the mouse population overestimated. In this example, rats really interfered with 95% of the blocks and mice 5%. When 100% of the blocks went missing, the technique simply did not work. Nevertheless, when census blocks remain in the treatment plot after application of toxic baits, and no (or little) gnawing is observed, this indicated a successful reduction in rat numbers.

The age and diphacinone content of the bait used in this study does not appear to have affected efficacy. The bait used in the wet forest in June 2000 was 13 months old and that in August 2000 nearly 16 months old. If this bait had a low acceptance to rats or a low concentration of diphacinone (which was not determined), then it could have allowed rats to survive. However, the bait disappeared rapidly from the forest floor (presumably eaten or cached by rats) in both June and August (personal observation), indicating that it was still attractive to rats. Also, bait from the same lot number used in the mesic forest in October 2000 (when nearly 18 months old) reduced rat abundance by 95% as measured by live trapping and 69% of the rats as measured by census blocks. Bait from the second lot number, used in the wet forest in December 2001 when 15 months old and with a diphacinone concentration of only 40.3 ppm, reduced rat abundance by 85–95%.

Our trapping efforts as part of this study documented all four species of non-indigenous rodents in both the wet and mesic forest study sites. Comprehensive surveys in nearby montane forests within Hawai'i Volcanoes National Park during the previous decade did not detect mice in wet forest or Norway rats in mesic forests (Scheffler *et al.* 2012). Thus, it appears that overall diversity of rodents may have increased within montane habitat in the park. This is important because of our success with reducing black rat populations and observations elsewhere of subsequent increases in the abundance of other rodent species (e.g., mice; Witmer *et al.* 2007). The LD_{50} of diphacinone for mice (50–300 mg/kg) is higher than for rats (0.3–7 mg/kg). Recent laboratory efficacy studies in Hawai'i using Ramik Green bait document lower mortality for mice compared to rats, but also higher bait acceptance (Pitt *et al.* 2011). House mouse abundance was reduced by our broadcast application of 50 ppm diphacinone bait, but not by as much as rat abundance. The influence of differences in bait acceptance, home range size, and competitive interactions among different rodent species within Hawaiian forests needs further investigation.

The recovery of a mongoose carcass with typical signs of anticoagulant poisoning and diphacinone residues in its livers and the discovery of green-dyed feces of mongooses and feral cats indicate that these species also may be affected by the broadcast application of Ramik Green bait containing 0.005% diphacinone for rat control. Further evidence of bait consumption by mongoose was provided by an adult male in another study, captured alive two weeks after application of Ramik Green bait, which contained 0.11 ppm diphacinone in its liver (unpublished data). The affected animals may have eaten bait directly or eaten rats or mice that had eaten bait. Diphacinone bait is registered for mongoose control, and the application of a different 0.005% diphacinone bait product (J. T. Eaton Corporation) in bait stations was highly effective in reducing mongoose abundance in Hamakua Marsh Wildlife Sanctuary (Smith *et al.* 2000). Radio telemetry and live trapping will be needed in future studies to determine the effects on mongoose abundance of broadcast application of Ramik Green bait.

Ground-based methods of applying diphacinone baits have recently proved successful for island-wide eradication of rats (Lujan *et al.* 2010, Hess and Jacobi 2011, Poncet *et al.* 2011). The results of this and previous studies in Hawai'i (Swift 1998, Dunlevy *et al.* 2000) indicate that broadcast application of 0.005% diphacinone bait can be highly effective for rat control in Hawaiian forests. However, the small size of the plots (4 ha) we were restricted to in this study allowed rapid reinvasion of rats. Aerial broadcast application of Ramik Green bait will allow larger areas to be treated in order to evaluate the effectiveness of this technique to control rat populations for the benefit of Hawai'i's endemic species.

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