Experimental Protocol for ContraPest Trial in Forest Areas

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6/15/17

**Purpose:** In order to protect endangered plant, bird and snail populations from the depredations of rats, we propose an experiment to determine whether ContraPest can be deployed effectively and safely in a forest setting. Our study addresses the following: 1. Does ContraPest reduce populations of *Rattus* spp. monitored by tracking tunnels, 2. Document with ink cards whether non-target visitors access the stations, and 3. Use histology to determine proportion of rats displaying reduced fertility.

**Problem Statement:** Rodents (*Rattus* spp. and *Mus musculus*) have been introduced to many ecosystems worldwide and are among the most widespread and problematic invasive animals affecting islands (Towns et al. 2006; Angel et al. 2009). Through mostly unintentional introductions by humans, these rodents occupy > 80% of the major islands worldwide (Atkinson 1985; Towns 2009). As a consequence of their omnivorous diet and large incisor teeth, introduced rats are probably the invasive animals responsible for the greatest number of plant and animal extinctions on islands (Towns et al. 2006).

Mesic forests are among the most diverse ecosystems in Hawaii, and many rare, threatened, or endangered plants, snails and insects reside in Hawaiian mesic forests. The U.S. Army is required to stabilize populations of endangered species and their habitat as per Biological Opinions issued by the U.S. Fish and Wildlife Service. Due to the large negative effects of introduced rats on natural resources at Kahanahaiki, which is an Army-managed 36-ha tract of mesic forest on the island of Oahu, the Oahu Army Natural Resources Program (OANRP) has been engaged in rodent control since 1995 using various techniques including snap traps, automatic traps, diphacinone rodenticide (the only approved rodenticide for use in conservation areas) applied in bait stations, and physical barriers. OANRP rat-control tools became more limited in 2012, which was when OANRP halted rodenticide use at all of the sites they manage (including Kahanahaiki) because of a change in the Special Local Needs (SLN) label that made bait-station application unfeasible in the steep, rugged terrain. Due to the high habitat quality and small size of Kahanahaiki, a large scale Victor Snap-trap grid of 402 traps was installed in May 2009 for ecosystem wide protection. In general, these traps were re-baited twice per month. After a general knock-down in the rat population in 2009, much rat activity fluctuation occurred and the targeted levels of rat suppression were not always being met with the large-scale snap-trapping (Pender et al. 2013); this resulted in noticeable losses of native and endangered seeds and predation of native snails by rats. During a trial in 2012 and 2013, Goodnature A24 rat + stoat traps (Goodnature Limited, Wellington, NZ), which are self-resetting traps that can function 24 times with one CO2 cartridge, were shown to be effective in controlling rat activity at a nearby site, Pahole gulch. Because of these results, a grid of A24s was installed at Kahanahaiki and snap-traps were discontinued. In July 2014, 83 Goodnature A24s were installed on existing trails at a spacing of 50 x 100 meters. In December 2014, an additional 36 A24s were installed within the gulch area to achieve a device spacing of 25 x 100 meters. In November 2015, a two-application (“one-time”) hand-broadcast of Diphacinone-50 according to label (Diphacinone 50: Conservation, EPA Reg. No.: 56228-35, State of Hawaii Lic. No. 8600.1) was conducted. The goal was to reduce the rat population (and therefore tracking) at Kahanahaiki during the seasonal
peak (roughly November-February), thereby improving conditions for the native and endangered species during this period.

Monitoring of rat activity at Kahanahaiki as well as a control site via tracking tunnels was implemented to determine efficacy of trapping devices (Figure 1). The OANRP management objectives for Kahanahaiki articulate that there should be less than 10% activity levels in rat tracking tunnels. An acceptable level of rat activity, which promotes stable or increasing native/endangered snail (*Achatinella mustelina*) and plant (*C. superba* subsp. *superba*) populations, has not been clearly identified, but New Zealand studies have shown that rat activity levels of 10% are low enough to maintain certain rare bird populations (Innes et al. 1999). A 10% activity level may also be the most achievable level using a large scale trapping grid. Results of the past seven years of monitoring of the control grid (May 2009-February 2017) show seasonal winter spikes of rat activity up to 78.4% (Figure 1). Therefore, relying solely on traps (snap-traps or A24s) has not been effective in keeping populations below the targeted 10% tracking in monitoring tunnels, particularly during the period of peak rat abundance (typically Fall/Winter; Figure 1).

**Figure 1.** Percent rat activity (based on tracking tunnels) at Kahanahaiki (the rat-trapping site), and two nearby sites where no rat trapping occurred (Pahole and Kapuna). The shaded area from November 2015-Present is when A24 traps were continued after a two application hand broadcast of Diphacinone 50 in November of 2015; July 2014-October 2015 is when only A24 traps were deployed; and the non-shaded (May 2009-April 2014) was when only Victor snap-traps were used.
**Study Site:** The Kahanahaiki Management Unit (MU) is located at 500-660 m elevation in the Waianae mountain range (21° 32’ N, 158° 11’ W), within the Makua Military Reservation (MMR), on Oahu, Hawaii. The total MU area is approximately 36 ha and is fenced to exclude ungulates. Overall, the north and east aspects are relatively native while the south and west exposures are dominated by weeds. Kahanahaiki is home to many rare taxa, including plants and snails; 15 plant species and two animals are listed as endangered (OANRP Staff, 2009). Non-native rodents are ubiquitous at Kahanahaiki, including black rats (*Rattus rattus*), Pacific rats (*R. exulans*), and house mice (*Mus musculus*); black rats are numerically dominant, outnumbering Pacific rats by >10-fold (Shiels 2010; Shiels and Drake 2011). Negative impacts of each of these three rodent species at Kahanahaiki has been reported to span native plants, insects, snails, and birds (Shiels et al. 2013). One endangered plant, *Cyanea superba*, is highly vulnerable to black rat predation, and large-scale and intensive snap-trapping at Kahanahaiki reduced seed predation by rats from 47% to just 4% in one season (Pender et al. 2013). Several additional native plants receive high predation by black rats at Kahanahaiki (Shiels and Drake 2011), implying that these native forests may potentially experience a shift in species composition attributable to invasive rats (particularly black rats).

**Methods:** For this trial two 4-hectare grids will be delineated at the Kahanahaiki management unit, one to be used as a control site and the other as the treatment site. The entire A24 grid will be discontinued and removed from the site for the duration of this trial. Localized control around rare resources just outside of the treatment area will be conducted when needed. Existing tracking tunnels will be maintained throughout the entire management unit. A grid of 25 ContraPest stations in JT Eaton 903TP tamper resistant bait stations (Figure 2) at a spacing of 50x50meters will be deployed over the 4-hectare (9.88 acre) treatment site (Figure 3). Within the control and treatment sites we will continue to monitor existing tracking tunnels as well as install an additional 14 tracking tunnels per site. A master control site located approximately 1 mile away where no rodent management has ever been conducted will also be monitored via tracking tunnels for comparison. Tracking tunnels will be monitored monthly at all sites.

A total of 12 monthly checks will be conducted starting August 2017 and continuing through July 2018. ContraPest stations will be re-baited with 1 liter of ContraPest per station (two 500ml containers) on a monthly interval and data will be recorded such as; amount of bait taken, any observations on the status/quality of bait, and non-target presence as evidenced by ink cards.

We feel the best thing to do will be to "bench" out/dig the dirt at each station site so it is level, we will then secure the stations with 2 metal 6" pegs attached through the holes near the two entrances and one metal 9" spike through the hole inside the station. The Management Unit is pig free and has an ungulate fence that is in working order and inspected every 3 months.

Tracking tunnel data will be analyzed using a Pearson’s chi-squared test (χ2) and results will be compared to the control site and historical tracking data.

At the conclusion of the trial period rodent trapping will be conducted at the control and treatment sites to collect tissue samples for histological examination of the reproductive system. Traps will be set and checked daily by OANRP staff. All animals will be weighed. Carcasses will be sampled and then burned on site. Ovaries will be trimmed of fat and weighed prior to being placed in 10% neutral buffered formalin for tissue fixation. The samples will be processed, paraffin-embedded and serially sectioned (5 μm), mounted and stained with hematoxylin and eosin; this will be conducted by trained SenesTech staff.
Follicles will be counted in every 40th section and classified as primordial, primary, secondary, or antral. Testes will be weighed and length and width documented.

Samples will be compared between the treated and control sections. Tracking tunnels will also be compared within the treatment and control sites as well as a master control site.

**Non-Target Concerns:** It is not anticipated that any native species will visit the bait stations or consume the ContraPest product.

**Deliverables:** Within 3 months of the conclusion of the field trial, we will produce a report on the efficacy of ContraPest to reduce rat activity relative to the control site. We will also compare its efficacy with other methods of rat control (traps and broadcast rodenticide). Any non-target impacts to other species will be noted. During each monthly check a carcass survey will be conducted on all of the trails looking for any non-target effects.

*Figure 2.* JT Eaton 903TP tamper resistant bait station with 500ml of ContraPest liquid bait inside station.

**Purchasing:**

We will be purchasing the product from SenesTech, Inc. We will be acquiring 300 liters of product total that will be shipped in batches from July 2017-June 2018.
Figure 3. Kahanahaiki management unit study site showing control (red grid) and treatment site (blue grid).


