2020

Laboratory Evaluation of the Effectiveness of the Fertility Control Bait ContraPest® on Wild-Captured Black Rats (*Rattus rattus*)

Shane Siers

Robert T. Sugihara

Israel Leinbach

Brandy R. Pyzyna

Gary Witmer

Laboratory Evaluation of the Effectiveness of the Fertility Control Bait ContraPest® on Wild-Captured Black Rats (*Rattus rattus*)

Shane R. Siers, **Robert T. Sugihara**, and **Israel L. Leinbach**
USDA APHIS, Wildlife Services, National Wildlife Research Center, Hawaii Field Station, Hilo, Hawaii
Brandy R. Pyzyna
SenesTech, Inc., Flagstaff, Arizona
Gary W. Witmer
USDA APHIS, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado

ABSTRACT: A non-toxic liquid fertility control bait for rats has recently become commercially available (ContraPest® from SenesTech, Inc.). This product contains two chemicals, both of which impair spermatogenesis in male and reduce ovulations in female rats. We tested the efficacy of this bait in wild-caught adult black rats from the island of Hawai'i in a short-term laboratory trial. A control group (n = 25) was offered placebo bait and the treatment group (n = 25) was offered fertility control bait, both *ad libitum*, during a 15-day introduction period and during the first of four breeding rounds, for a total of 58 days of exposure. After treatment, all rats were provided placebo bait for the remainder of the study and randomly paired with mates from within their treatment groups for two additional breeding cycles. Treatment and control groups comprised 10 breeding pairs each, with random re-pairings between breeding rounds. The treatment group produced no litters during the first and second breeding rounds, while 70% of the control females produced litters. In the third breeding round, 70 days after stopping treatment, the treatment group produced three litters (six pups) compared to seven litters (24 pups) in the control group. During a fourth and final breeding round, control rats were crossed with treated rats, producing six litters (27 pups) from treated dams and nine litters (40 pups) from control dams, indicating no apparent infertility effect 99 days after cessation of treatment. This study demonstrates that the reproduction rate of wild-caught black rats can be chemically suppressed if provided *ad libitum* access to the fertility control bait under laboratory conditions.

KEY WORDS: 4-vinylcyclohexene diepoxide, black rat, contraception, fertility, laboratory efficacy, *Rattus rattus*, reproductive inhibition, triptolide, VCD

Proceedings, 29th Vertebrate Pest Conference (D. M. Woods, Ed.)
Paper No. 50. Published December 10, 2020. 7 pp.

INTRODUCTION

Invasive mammals impose great environmental and economic costs through damage to agricultural crops, natural resources, and human health and safety (Bergman et al. 2000, Pimentel et al. 2005). Invasive mammals can be particularly devastating on islands and have led to considerable species decline and extinctions (Courchamp et al. 2003, Doherty et al. 2016), with rats being one of the most damaging taxa of invader (Jones et al. 2008, Varnham 2010). In addition to the damage they cause in natural environments, invasive pest rodents have caused severe human and economic costs through damage to agriculture contributing to famine, vectoring zoonotic diseases, and mechanical damage resulting from gnawing and nesting behavior (Singleton et al. 2010, Himsworth et al. 2013). In Hawai'i, invasive black rat (*Rattus rattus*) populations damage crops and food stores, kill native flora and fauna, and are reservoirs and vectors of human disease (Meerburg et al. 2009, Shiels et al. 2014), including leptospirosis and rat lungworm disease (Jarvi et al. 2014, 2015, 2017).

In addition to traditional components of integrated pest management such as sanitation (removal of food sources), habitat management, and physical exclusion, large-scale rat control in protection of agriculture, human heath, and natural resources has typically involved the use of rodenticides: lethal toxicants formulated into an attractive and palatable bait matrix (Hadler and Buckle 1992, Buckle 1999, Witmer et al. 2007, Witmer and Eisemann 2007, Buckle and Smith 2015). However, shifting societal values (e.g., in opposition to perceived risk of poisoning of nontarget wildlife, animals and children) are increasing the demand for non-toxic, non-lethal alternatives for resolution of human-wildlife conflicts. Traditional anticoagulant rodenticides have aroused concern over poisoning of non-target species, environmental contamination, and humaneness (Mason and Littin 2003, Eason et al. 2010). Wildlife fertility control has been considered as a potential long-term management approach for reducing pest populations and the damage they cause (Miller at al. 1998). Fertility control has been predicted to prevent the rebound of rodent populations seen after rodenticide application (Gao and Short 1993) by reducing the rate of reproduction following temporary release from density-dependent population regulation (Jacob et al. 2008).

SenesTech, Inc. (Flagstaff, Arizona) markets a U.S. Environmental Protection Agency-registered, commercial liquid bait formulation, ContraPest®, for fertility control in rats. It contains two active ingredients that target both follicle development and spermatogenesis, blocking reproduction in both sexes. The active ingredient 4-vinylcyclohexene diepoxide (VCD) causes primordial follicle depletion leading to premature ovarian failure (Hoyer et al. 2001, Mayer et al. 2002, Mayer et al. 2004, Mark-Kappeler 2011). Follicular maturation progresses from the primordial stage to primary, secondary, antral and preovulatory in preparation for ovulation (Mayer et al. 2002). VCD targets the finite pool of primordial follicles;

once depleted, and after the remaining follicle types have been eliminated by atresia or ovulation, ovarian function is terminated (Hoyer et al. 2001, Mayer et al. 2004, Jacob et al. 2008, Mauldin 2013). VCD causes primordial follicle loss by interfering with KIT signaling, a key cellular growth and survival pathway within the oocyte (Mark-Kappeler et al. 2011). Atresia is a natural process in the ovary to eliminate follicles not destined for ovulation. VCD greatly accelerates this natural process (Hoyer at al. 2001). The second active ingredient in ContraPest is triptolide, a diterpene triepoxide purified from the traditional Chinese medicinal plant *Tripterygium wilfordii*. Triptolide stops growing follicles in the ovary and sperm production in the testes (Lue et al. 1998, Huynh et al. 2000, Xiong et al. 2011, Zeng et al. 2017). ContraPest has very low concentrations of both actives, VCD at 0.09% and triptolide at 0.001%. The combination of these two active ingredients acts synergistically to suppress reproduction in both sexes. Witmer et al. (2017) recently tested the palatability and efficacy of ContraPest fertility control bait in both Sprague-Dawley laboratory rats and in wild-caught Norway rats (*R. norvegicus*). Sprague-Dawley rats were provided *ad libitum* access to the liquid bait, along with *ad libitum* chow and water for 21 days. Rats that took treatment bait were placed in breeding pairs, as were control rats that took bait without active ingredients. Rats that received treatment bait had no offspring, while 100% of control rats had litters after one breeding round. Similar results of no offspring were found in breeding pairs of wild-caught Norway rats, tested in the laboratory, which took treatment bait and then completed two breeding rounds (Witmer et al. 2017).

ContraPest has yet to be tested on black rats, the species with the most widespread impacts on island ecosystems (Jones et al. 2008, Shiels et al. 2014). To date, there are no reports on the impact of the combination of ContraPest's two active ingredients on black rats, nor has the impact of VCD on their fertility been reported. Of the two active ingredients, only triptolide's impact on male black rat fertility has been studied (Singla et al. 2013, Singla and Challana 2014). In Singla and Challana (2014), the reproductive toxicity of triptolide was examined in no choice feeding at 0.1%, 0.2%, or 0.3% for 5 days with wild-caught male black rats. After 15 days post-dosing, the treated male rats were mated with healthy, untreated and cyclic female rats for 15 days. Only the male rats that took 0.2% triptolide sired no pups which may have been due to significantly reduced sperm motility and viability (Singla and Challana 2014). When the concentrations of triptolide taken by wild-caught brown rats versus wild-caught black rats are compared, taking into account the difference in percent concentrations in the two different baits, average consumption for each male rat, and difference in body weights between the larger brown versus black rats, the male black rats were exposed to 1,376 times the amount of triptolide taken by choice by the male brown rats. No mortalities were reported for either black or brown male rats in these two studies (Dyer et al. 2013, Singla and Challana 2014). Given the large difference in triptolide concentration that induced infertility in male black versus male brown rats, we sought to determine what impact ContraPest would have on the fertility of wild-caught black rats. Perhaps ContraPest would be ineffective in black rats due to their apparent insensitivity to triptolide, as over a 1000-fold greater triptolide was needed to suppress male rat fertility. Because ContraPest is presented in bait stations which both male and female black rats would visit, we presented ContraPest to both sexes to determine the impact on their fertility and fecundity.

METHODS

Animal Acquisition, Preparation, and Disposition

Wild black rats were live-trapped in forests and other conservation areas near Hilo and Volcano, County of Hawaii. In addition to trapping at our own USDA Hilo facility, permissions for trapping were granted by the Environmental Office of the Keaukaha Military Reservation, the site manager of Mauna Loa Orchards, and private owners of residential properties in the village of Volcano. Captured rats were transported to the testing facility and dusted with Drione® insecticide (Bayer, Research Triangle Park, NC) to treat for ectoparasites before being housed. Fifty rats of equal sex ratio were housed individually in numbered metal laboratory cages in a climate- and lighting-controlled laboratory space at the testing facility (20-22°C, ambient humidity, and 12 hr on/off light cycle). Cages (22 cm × 57 cm × 19 cm) were furnished with PVC refuge tubes sized for one or two rats (isolation or breeding events) and commercially purchased shredded paper bedding, replaced as needed. All rats had unrestricted access to a maintenance diet of Purina® rodent chow pellets (Nestle Purina PetCare Company, St. Louis, MO), and water was provided *ad libitum* in 250-ml inverted glass bottles with stainless steel sipper tubes throughout the duration of the study. Rats also received wood chew sticks with replacement as necessary.

All rats were individually housed for a minimum quarantine period of 3.5 weeks to ensure that no females were pregnant at the outset of the study phase. Rats were weighed at the beginning of the quarantine period, prior to pairing, and again at the end of the trial phase. All young born during the study were removed upon parturition and euthanized via an overdose of inhalant anesthesia (isoflurane) with subsequent carbon dioxide (CO_2) immersion. Adult rats were euthanized via CO_2 overdose at the end of the study. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and within the terms of the study protocol approved by the Institutional Animal Care and Use Committee of the U.S. Department of Agriculture National Wildlife Research Center (QA-2570).

Statistical Analyses

All statistical analyses and data visualization were performed in the R language for statistical computing (R Core Team 2016). Specific functions and tests are described within the methods subsections below.

Bait Consumption

Liquid ContraPest, containing the active ingredients VCD and triptolide (i.e., active bait), or an identical formulation lacking the active ingredients (i.e., placebo bait), was offered *ad libitum* in identical 250-ml inverted

glass bottles, as were the bottles providing water. Daily bait consumption was estimated by measuring the bait level within the bottles with a graduated scale. While co-housed for breeding we continued to record bait consumption, though we were unable to determine how much bait was consumed by each individual. To test for an effect of the active ingredients on bait consumption (i.e., palatability effects of VCD and triptolide), data from the initial exposure and first breeding cycle phases (the period during which the treatment group received the active bait) were subjected to a linear mixed effects model with bait type (active vs. placebo), sex, and study phase (active bait exposure vs. pre- and post-exposure periods) as fixed effects and individual identification (ID) as a random effect (1|ID) to account for multiple repeated measures for each individual. Modeling was conducted using the function "lmer" in the package "lme4" (Bates et al. 2015), with the model specified as:

$$\text{consumption} \approx \text{bait} + \text{sex} + \text{phase} + (1|\text{ID})$$

To obtain a p-value for the effect of bait type on consumption, we performed a likelihood ratio test comparing this model to a null model without the bait term in an analysis of variance (ANOVA); the p-value for the χ^2 comparison of the two models is reported as the statistical significance of the bait effect.

Reproductive Inhibition Trials

Prior to pairing for breeding, all rats were pretreated with the placebo bait formulation for a five-day conditioning period to ensure that rats were familiar with the bait prior to the treatment period (trial Days -6 to -1). Within each sex group, 13 rats were randomly assigned to the active bait treatment group and 11 females and 13 males were assigned to the placebo bait control group. After the conditioning period, the treatment group was administered the active bait for 15 days while the control group continued to receive the placebo bait (Days 0 to 15). Weight, sex, cage number, and treatment group assignment of each pair was recorded before the initiation of the breeding cycles.

During the first of four breeding rounds (Round 1, Days 15 to 35), the treatment and control groups continued receiving active and placebo bait, respectively. Ten females were randomly paired with ten males within their respective study groups (treated females paired with treated males, control females paired with control males) and the males were placed within the females' cages for mating, with individual IDs recorded for each pairing. The remaining rats in each group continued to be housed individually, to be substituted for rats found to be unfit for breeding due to poor condition, injury, or rejection of a male by the female partner during the course of the breeding cycle.

Males were paired with females for 21 to 23 days. If a male was rejected by the female within 24 to 48 h, one of the spare males from the same study group (treatment or control) was substituted for breeding. Females and/or cage papers were examined daily for discarded vaginal plugs as an indication that they had been inseminated. After the pairing period, males were removed and returned to their individual cages. Females were monitored for parturition daily for 23-28 days following removal of males. Within 24 h of birth, pups were removed, counted, and euthanized.

At the completion of the first breeding cycle, the active bait provided to the treatment group was withdrawn and replaced with the placebo bait to determine the persistence of a reproductive inhibition effect (Day 58). At this time, the treatment group had been continuously exposed to the active bait for 58 days. All rats were provided the placebo bait for the remainder of the study.

For a second and third breeding cycle (Rounds 2 and 3, Days 58 to 79 and 105 to 127), pairings within study groups were re-randomized without replacement so that males were placed with different females than in previous breeding cycles. For a fourth and final breeding cycle (Round 4, Days 156 to 177), females from the treatment group were crossbred with males from the untreated control group, and treated males were paired with un-treated females in order to assess whether treatment of a single sex suppressed reproduction.

After the last round of breeding, all animals were euthanized and body weights recorded. Liver, kidneys (combined), spleen, adrenal glands (combined), and reproductive organs were excised, cleaned of fats and/or connective tissues, and weighed for future comparative analysis.

Statistical differences between counts of litters for treatment and control groups, per breeding round, were tested with Fisher's exact tests Wilcoxon rank tests, with α set at 0.05 and two-tailed p-values reported. These methods were not intended to distinguish between a contraceptive effect on males or females.

RESULTS

Bait Consumption

Daily bait consumption by sex, study group, and study phase is depicted in Figure 1. All rats readily consumed the bait. The median rat (ranked by consumption) consumed an average of 74 ml of bait per day; individual means ranged from 66 to 83 ml per day. There was no effect of inclusion of the active ingredients in the bait formulation on bait consumption (i.e., there was no apparent negative effect of active ingredients on palatability; study phase term $p = 0.739$).

During this project, males (which grow larger than females) gained significant weight, but there was no discernible difference in weights between test groups of males or females receiving placebo or active-ingredient baits during the treatment phase (Figure 2).

Reproductive Inhibition Trials

During pairings, there were only two occasions when males were removed and replaced due to incompatibility/ aggression by females. Pairings and litter size details for all four breeding rounds are tabulated in Siers et al. (2017).

Numbers of litters and pups per litter for each of the ten breeding pairs per round are summarized in Table 1. During the first breeding round, when the treatment group had been exposed to active ContraPest bait for 15 days and was continuing to consume the active bait, there were no litters within the treatment group, while seven litters, totaling 32 pups, were born to the 10 control pairs (70% breeding success). During the second breeding round, which began simultaneously with the replacement of the

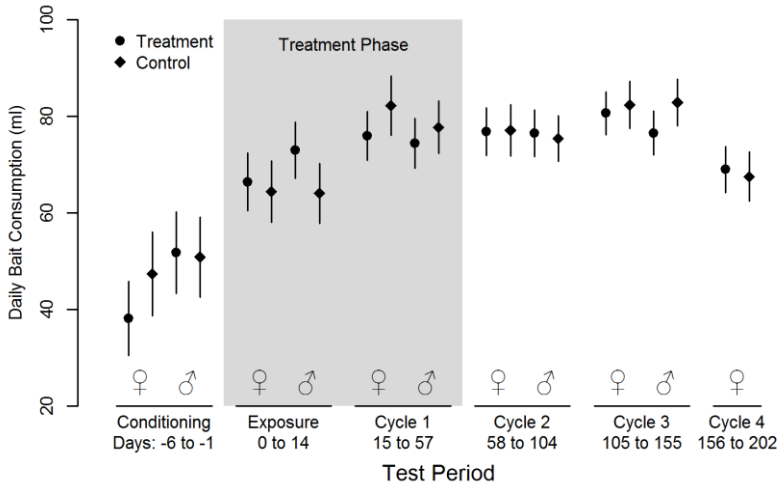

Figure 1. Bait consumption. Mean daily bait consumption and 95% confidence intervals (1.96*SE) by sex, study group, and study phase. Bait including the active ingredient was only offered during the Treatment Phase. Individual consumption data were only available while rats were individually-house (not while paired for breeding). There is no Cycle 4 consumption data for males because they were euthanized immediately following breeding. N = 11 for the female control group, N = 13 for all other groups.

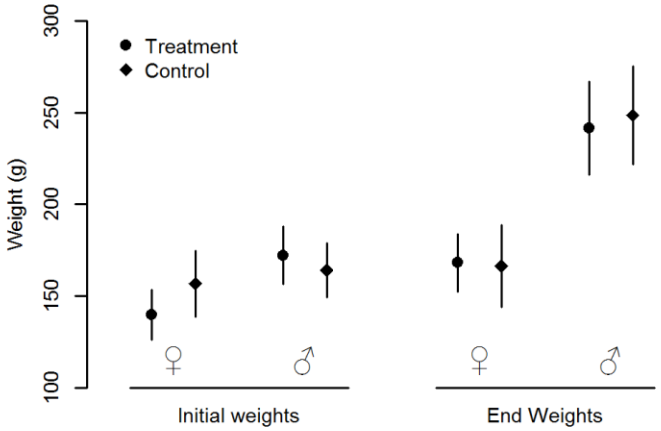

Figure 2. Body weights. Mean rat body weights and 95% confidence intervals (1.96*SE) by sex and study group. Within sexes, there were no significant weight differences between treatment and control groups (α = 0.05). N = 11 for the female control group and N = 13 for all other groups.

Table 1. Litter count and litter size results for female rats (N = 10 per study group). "Bait" indicates whether the treatment group was provided either the active ingredient ContraPest product or the placebo version during the breeding cycle. "Mating" denotes whether females were matched to males by study group (treatment-treatment/control-control) or crossed with males of the opposite study group (treatment-control/control-treatment). "Mean Litter" size is calculated only from females with litters (zeroes not included in the average); however, the Wilcoxon rank test for difference in litter size is based on a litter size of zero for dams without litters. * p ≤ 0.05; ** p ≤ 0.01.

Breeding Round	Days Paired	Bait	Mating	Litters		Pups		Mean Litter ± SD	
				Ctrl	Trt	Ctrl	Trt	Ctrl	Trt
1	15-35	Active	Matched	7	0**	32	0	4.57 ± 1.05	0**
2	58-79	Placebo	Matched	7	0**	27	0	3.86 ± 2.17	0**
3	105-127	Placebo	Matched	7	3	24	6	3.43 ± 1.18	2.00 ± 1.41*
4	156-177	Placebo	Crossed	9	6	40	27	4.44 ± 2.91	4.50 ± 2.22

treatment group's active bait with placebo, there continued to be no reproduction in the treatment group pairs and 70% breeding success (seven litters, 27 pups) in the control group. During both first and second breeding rounds, the difference between seven control litters and zero treatment litters was statistically significant ($p = 0.004$). By the beginning of the Round 3, the active bait had been replaced by placebo for 47 days. During this round, control group reproduction remained at 70%, while treatment group reproduction increased to 30%; though continued reproductive suppression is apparent, the difference between control and treatment group litters was not statistically significant at $\alpha = 0.05$ ($p = 0.178$). Considering only females that produced litters, the litter sizes in the treatment group (four, one, and one) were smaller than those in the control group ($p \leq 0.05$). By the fourth round of breeding, commencing 99 days after removing active bait, treatment group females paired with control group males reproduced at a rate indistinguishable from control group fecundity in previous rounds. There was no apparent suppressive effect when mating control group females with treatment group males, with this group producing the most litters (90% breeding success) and bearing litter sizes indistinguishable from previous control female breeding rounds.

DISCUSSION

These results demonstrate complete reproductive inhibition for wild-caught black rats exposed to ContraPest bait containing the active ingredients VCD and triptolide, *ad libitum*, under laboratory conditions, for at least 15 consecutive days prior to mating and throughout a 43-day breeding cycle. The inhibitive effect persisted through the second breeding cycle. When paired a third time, 47 days after cessation of treatment, a partial suppressive effect was apparent but not statistically significant, though litter sizes were significantly smaller for the few treatment females that did reproduce. By 99 days post-treatment (Round 4) there was no apparent effect of reproductive inhibition. Given that fertility was rebounding by the third breeding cycle, we are unable to draw any useful inference from the cross-breeding of treated and control animals, and the ability to detect any potential sex-specific effect is confounded by the dissipation of the treatment effect. The impact of the two active ingredients of ContraPest caused infertility for a length of time that exceeded the spermatogenic cycle reported by Singla et al. (2013); however, our study design did not allow for discrimination between a contraceptive effect on males or females. In practice, both sexes will receive the bait, and based on prior research cited in the introduction we expect that both sexes are affected to some degree.

Reproduction within our control group was not 100%. During the first three breeding cycles, three (30%) of the control group pairings did not resulted in litters, and one (10%) of the parings in the fourth cycle did not produce a litter. This could potentially be a result of failure of wild-caught rats to fully adjust to captivity in a relatively short timeframe, or an indication of incomplete fertility in the source population from which these individuals were drawn. While differences in litters and litter sizes were statistically significant during the first two breeding rounds, without 100% reproduction in the control group it is difficult to argue that the lack of any litters in the first two breeding rounds was solely attributable to the ContraPest treatment and with no influence of sub-fertility in the treatment group.

By design, ContraPest is a contraceptive and not a sterilant. As a result, no evidence of permanent infertility was detected following the 58-day active bait exposure period. Whether more prolonged exposure to ContraPest would lead to permanent sterilization cannot be inferred from our study. Further studies would be needed to assess the effect of long-term exposure on fertility.

Refinements of this or other fertility control baits might afford non-toxic and non-lethal alternatives for protection of agriculture, human health and safety, and natural resources under some management scenarios. For instance, ContraPest could complement conventional use of toxicants that have caused a rapid knockdown of the pest population; following up with the use of ContraPest could prevent the well-known rebound of a poisoned rat population (Andrews 1977). Timing the use of ContraPest would be key to diminishing rat population expansion due to seasonal weather changes and/or abundance of food sources. In Hawai‘i, for example, ContraPest could be considered to suppress the marked increase in rat population that follows the peak of strawberry guava fruiting by two to three months (Shiels 2010).

ContraPest has also undergone successful trials for reducing brown rat population levels in the New York City Subway system in 2013 (Klein 2017). Because brown rats in subway trash rooms chose liquid when given choice between solid and liquid matrices to deliver the active ingredients, ContraPest is currently formulated as a liquid. For treatment of black rats in challenging tropical terrains, where deployment of bait boxes with liquid tanks is not feasible, it will be necessary to develop and evaluate a solid form of ContraPest that can be aerially deployed and is durable in the tropical environment. This study demonstrates that the active ingredients in ContraPest, when ingested *ad libitum* in the current liquid formulation, can profoundly reduce black rat reproductive output.

ACKNOWLEDGEMENTS

Tracey Borneman, Dean Foster, Lauren Jaebker, Tiana Maple, Cynthia Payne, and Celeste Samra (USDA) assisted with lab trials. Kaitlyn Jacobs (USDA) drafted introductory matter, and valuable edits were offered by Dana Skarra (SenesTech, Inc.). John Eisemann (USDA) coordinated the Cooperative Research and Development Agreement between SenesTech, Inc., and USDA. Cheryl Dyer and Loretta Mayer (SenesTech, Inc.) assisted with project design and provided valuable edits to earlier versions of this manuscript. This work was supported in part by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center. Reference herein to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government.

LITERATURE CITED

Andrews, R. V. 1977. Should we kill rats or is biological control preferable? Transactions of the Nebraska Academy of Sciences and Affiliated Societies 448:203-207.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1-48.

Bergman, D. L., M. D. Chandler, and A. Locklear. 2000. The economic impact of invasive species to Wildlife Services' cooperators. Pages 169-184 *in* L. Clark, editor. Proceedings, 3rd National Wildlife Research Center Special Symposium. National Wildlife Research Center, Fort Collins, CO.

Buckle, A. P., and R. H. Smith. 2015. Rodent pests and their control. Center for Agriculture and Bioscience International (CABI), Oxfordshire, United Kingdom.

Buckle, A. P. 1999. Rodenticides: their role in rodent pest management in tropical agriculture. Pages 163-177 *in* G. R. Singleton, L. A. Hinds, H. Leirs, and Z. Zhang, editors. Ecologically-based management of rodent pests. ACIAR Monograph 59:163-177.

Courchamp, F., J. Chapuis, and M. Pascal. 2003. Mammal invaders on islands: impact, control and control impact. Biological Reviews 78:347-383.

Doherty, T. S., A. S. Glen, D. G. Nimmo, E. G. Ritchie, and C. R. Dickman. 2016. Invasive predators and global biodiversity loss. Proceedings of the National Academy of Sciences 113(40):11261-11265.

Dyer, C. A., S. Raymond-Whish, S. Schmuki, T. Fisher, B. Pyzyna, A. Bennett, and L. P. Mayer. 2013. Accelerated follicle depletion in vitro and in vivo in Sprague-Dawley rats using the combination of 4-vinylcyclohexene diepoxide and triptolide. Journal of Zoo and Wildlife Medicine 44:S9-S17.

Eason, C. T., K. A. Fagerstone, J. D. Eisemann, S. Humphrys, J. R. O'Hare, and S. J. Lapidge. 2010. A review of existing and potential New World and Australasian vertebrate pesticides with a rationale for linking use patterns to registration requirements. International Journal of Pest Management 56:109-125.

Gao, Y., and R. V. Short. 1993. The control of rodent populations. Oxford Review of Reproductive Biology 15:265-310.

Hadler, M. R., and A. P. Buckle. 1992. Forty-five years of anticoagulant rodenticides: past present and future trends. Proceedings of Vertebrate Pest Conference 15:149-155.

Himsworth, C. G., K. L. Parsons, C. Jardine, and D. M. Patrick. 2013. Rats, cities, people, and pathogens: a systematic review and narrative synthesis of literature regarding the ecology of rat-associated zoonoses in urban centers. Vector-borne and Zoonotic Diseases 13:349-359.

Hoyer, P. B., P. J. Devine, X. Hu, K. E. Thompson, and I. G. Sipes. 2001. Ovarian toxicity of 4-vinylcyclohexene diepoxide: a mechanistic model. Toxicologic Pathology 29(1):91-99.

Huynh, P. N., A. P. S. Hikim, C. Wang, K. Stefonovic, Y. H. Lue, A. Leung, V. Atienza, S. Baravarian, V. Reutrakul, and R. S. Swerdloff. 2000. Long-term effects of triptolide on spermatogenesis epididymal sperm function, and fertility in male rats. Journal of Andrology 21:689-699.

Jacob, J., G. R. Singleton, and L. A. Hinds. 2008. Fertility control of rodent pests. Wildlife Research 35:487-493.

Jarvi, S., W. Pitt, A. Osuna, M. Farias, L. Shiels, K. Howe, S. Jacquier, A. Shiels, K. Amano, B. Luiz, and D. Maher. 2014. Efficacy of a vaccine for *Angiostrongylus costaricensis* against rat lungworm disease caused by *A. cantonensis* in wild Hawaiian rats (*Rattus rattus*). Journal of Immunology 192(1 Supplement):141.123.

Jarvi, S. I., W. C. Pitt, M. E. Farias, L. Shiels, M. G. Severino, K. M. Howe, S. H. Jacquier, A. B. Shiels, K. K. Amano, B. C. Luiz, and D. E. Maher. 2015. Detection of *Angiostrongylus cantonensis* in the blood and peripheral tissues of wild Hawaiian rats (*Rattus rattus*) by a quantitative PCR (qPCR) assay. PLoS ONE. 10:e0123064.

Jarvi, S. I., S. Quarta, P. Eamsobhana, K. Howe, S. Jacquier, R. McHugh, K. Kramer, and M. Meyers. 2017. Human exposure to *Angiostrongylus cantonensis* on east Hawaii Island. Journal of Immunology 198(1 Supplement):57.16.

Jones, H. P., B. R. Tershy, E. S. Zavaleta, D. A. Croll, B. S. Keitt, M. E. Finkelstein, and G. R. Howald. 2008. Severity of the effects of invasive rats on seabirds: a global review. Conservation Biology 22:16-26.

Kao, S., I. G. Sipes, and P. B. Hoyer. 1999. Early effects of ovotoxicity induced by 4-vinylcyclohexene diepoxide in rats and mice. Reproductive Toxicology 13:67-75.

Klein, A. 2017. Menopause-causing bait is curbing rat populations in New York. New Scientist. 5 May 2017.

Lue, Y., A. P. Sinha Hikim, C. Wang, A. Leung, S. Baravarian, V. Reutrakul, R. Sangsawan, S, Chaichana, and R. S. Swerdloff. 1998. Triptolide: a potential male contraceptive. Journal of Andrology 9:479-486.

Mark-Kappeler, C. J., N. Sen, A. Lukefahr, L. McKee, I. G. Sipes, J. Konhilas, and P. B. Hoyer. 2011. Inhibition of ovarian KIT phosphorylation by the ovotoxicant 4-vinylcyclohexene diepoxide in rats. Biology of Reproduction 85:755-762.

Mason, G., and K. E. Littin. 2003. The humaneness of rodent pest control. Animal Welfare 12:1-37.

Mauldin, R. 2013. 4-Vinyl-1-cyclohexene diepoxide and ERL 4221-induced oocyte depletion in the juvenile female Norway rat ovary. Final Report QA-1431. USDA APHIS, WS, National Wildlife Research Center, Fort Collins, CO.

Mayer, L. P., P. J. Devine, C. A. Dyer, and P. B. Hoyer. 2004. The follicle-deplete mouse ovary produces androgen. Biology of Reproduction 71:130-138.

Mayer, L. P., N. A. Pearsall, P. J. Christian, P. J. Devine, C. M. Payne, M. K. McCuskey, S. L. Marion, I. G. Sipes, and P. B. Hoyer. 2002. Long-term effects of ovarian follicular depletion in rats by 4-vinylcyclohexene diepoxide. Reproductive Toxicology 16:775-781.

Meerburg, B. G., G. R. Singleton, and A. Kijlstra. 2009. Rodent-borne diseases and their risks for public health. Critical Reviews in Microbiology 35:221-270.

Miller, L. A., B. A. Johns, and D. J. Elias. 1998. Immunocontraception as a wildlife management tool: some perspectives. Wildlife Society Bulletin 26:237-243.

Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52:273-288.

R Core Team. 2016. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Shiels, A. B. 2010. Ecology and impacts of introduced rodents (*Rattus* spp. and *Mus musculus*) in the Hawaiian Islands. Ph.D. dissertation, University of Hawaii, Manoa, HI.

Shiels, A. B., W. C. Pitt, R. T. Sugihara, and G. W. Witmer. 2014. Biology and impacts of Pacific Island invasive species. 11: *Rattus rattus*, the black rat (Rodentia: Muridae). Pacific

Science 68:145-184.
Siers, S. R., B. R. Pyzyna, L. Mayer, C. Dyer, I. L. Leinbach, R. T. Sugihara, and G. W. Witmer. 2017. Laboratory evaluation of the effectiveness of the fertility control bait ContraPest® on wild-captured black rats (*Rattus rattus*). Unpublished Report QA-2570. USDA APHIS, WS, National Wildlife Research Center, Hilo, HI.
Singla, N., and S. Challana. 2014. Reproductive toxicity of triptolide in male house rat, *Rattus rattus*. The Scientific World Journal 2014: article 879405.
Singla, N., G. Kaur, B. K. Babbar, and B. S. Sandhu. 2013. Potential of triptolide in reproductive management of the house rat, *Rattus rattus*. Integrative Zoology 8:260-276.
Singleton, G. R., S. R. Belmain, P. R. Brown, and B. Hardy, editors. 2010. Rodent outbreaks: ecology and impacts. International Rice Research Institute, Manila, Philippines.
Varnham, K. 2010. Invasive rats on tropical islands: their history, ecology, impacts and eradication. RSPB Research Report No. 41. Royal Society for the Protection of Birds, Bedfordshire, United Kingdom.
Witmer, G., and J. D. Eisemann. 2007. Rodenticide use in rodent management in the United States: an overview. Proceedings of Wildlife Damage Management Conference 12:114-118.
Witmer, G., J. D. Eisemann, and G. Howald. 2007. The use of rodenticides for conservation efforts. Proceedings of Wildlife Damage Management Conference 12:160-167.
Witmer, G. W., S. Raymond-Whish, R. S. Moulton, B. R. Pyzyna, E. M. Calloway, C. A. Dyer, L. P. Mayer, and P. B. Hoyer. 2017. Compromised fertility in free feeding of wild-caught Norway rats (*Rattus norvegicus*) with a liquid bait containing 4-vincylcyclohexene diepoxide and triptolide. Journal of Zoo and Wildlife Medicine 48:80-90.
Xiong, J., H. Wang, G. Guo, S. Wang, L. He, H. Chen, and J. Wu. 2011. Male germ cell apoptosis and epigenetic histone modification induced by *Tripterygium wilfordii* Hook F. PLoS ONE 6:e20751.
Zeng, Y., H. Sun, Y. Li, M. Shao, P. Han, X. Yu, L. He, Y. Xu, and S. Li. 2017. Exposure to triptolide affects follicle development in NIH mice: role of endoplasmic reticulum stress in granulosa cell apoptosis. Human and Experimental Toxicology 36:82-92.