



U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Wildlife Services

U.S. Government Publication

Free Ranging Wild-Caught Norway Rats Have Reduced Fecundity after Consuming Liquid Oral Fertility Bait Containing 4-Vinylcyclohexene Diepoxide and Triptolide

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ABSTRACT: Norway rats cause extensive crop loss, infrastructure damage, and are vectors for zoonotic diseases. Due to growing efficacy, environmental, and animal welfare concerns related to traditional pest management tools, it is important to find new methods for controlling commensal rodents. Fertility control is emerging as a safe, humane, and effective method of long-term pest population management. SenesTech Inc. has developed an oral, liquid fertility management bait with two active ingredients: 4-vinylcyclohexene diepoxide (VCD) and triptolide. Previously, a 95% reduction in pups was observed through three breeding rounds when wild-caught Norway rats were caged in pairs and offered this bait for 50 days. Following these results, wild-caught Norway rats ($n=6$ males; $n=15$ females per group) were placed in open arenas, offered bait (in the presence of rat chow and water) *ad libitum* for 56 days, and allowed to breed for four rounds. Animals were bred within their treatment paired groups (control or treatment) for the first three rounds and then treatment cross-bred during the fourth round. Through three breeding rounds, 255 pups were born to control breeding pairs, compared to 12 pups born to treated breeding pairs. In the final round, 93 pups were born to control females paired with treated males and 80 pups were born to treated females paired with control males. A significant reduction in epididymis weight, and in testis weight and volume, was observed in treated males, while ovarian weight was reduced in treated females. These results indicate that fertility was dramatically reduced in wild caught Norway rats after consuming fertility management bait. Rats voluntarily consumed the treatment bait, and this free selection is essential for future field trials where the ability of the bait to reduce wild rat populations will be assessed in agricultural and urban settings.

KEY WORDS: 4-vinylcyclohexene diepoxide, fertility control, Norway rat, *Rattus norvegicus*, triptolide

Proc. 27th Vertebr. Pest Conf. (R. M. Timm and R. A. Baldwin, Eds.)
Published at Univ. of Calif., Davis. 2016. Pp. 314-316.

INTRODUCTION

Commensal rodents are a persistent problem for humans worldwide. They can cause food shortages, damaged property and infrastructure, and are vectors for numerous diseases (Witmer 2007, Witmer and Singleton 2010). In the U.S. alone, it is estimated that rats are the source of \$19 billion in losses per year (Parsons et al. 2015). While rodenticides have been a mainstay in controlling rodent populations in many situations, they may be effective only in the short-term (Witmer and Eisemann 2007, Witmer and Pitt 2012).

Rodenticide baiting often leads to rapid population rebounds which may even exceed the original population size (Knipling and McGuire 1972). Rebounds are driven by increased survival and breeding success of remaining animals, as well as re-invasion from surrounding areas (Singleton et al. 2007, Witmer and Singleton 2010). Additionally, rodenticides may become less effective over time (e.g., genetic or behavioral resistance), may be more restricted in their use, or may be removed from the commercial market (Buckle and Prescott 2012). There are also growing concerns about the humaneness and non-target hazards posed by rodenticides. Alternatives to reduce rodent populations and damage are required.

Fertility control is an alternative strategy to rodenticides for controlling commensal rodent populations and damage caused by rodent infestations (Knipling and McGuire 1972, Gao and Short 1992, Jacob et al. 2008).

Prohibiting reproduction through fertility management can decrease population growth while retaining reproductively compromised individuals within the population to compete with fertile animals for food, water, space, and mates. Fertility management bait must be highly palatable to attract rodents away from natural food sources and to ensure consumption of effective doses (Knipling and McGuire 1972, Gao and Short 1992).

In an attempt to establish more humane approaches to control overabundant rodents, SenesTech, Inc. (Flagstaff, AZ) has developed a liquid fertility management bait, ContraPest[®], which contains two active ingredients that target follicle loss: 4-vinylcyclohexene diepoxide (VCD) and triptolide. VCD accelerates the natural process of atresia in primordial follicles (Mayer et al. 2002, Mayer et al. 2004, Kanter et al. 2006). Triptolide promotes apoptosis in secondary follicles (Xu and Zhao 2010), and leads to infertility in male rats by disrupting spermatogenesis (Hikim et al. 2000, Huynh et al. 2000).

ContraPest[®] has been shown to be palatable to free living wild rats (Pyzyna et al. 2014) and decreased offspring numbers in both laboratory (Dyer and Mayer 2014) and wild rats in captivity (Witmer et al. 2017). An open arena breeding study was conducted at the USDA National Wildlife Research Center (NWRC) in Fort Collins, CO. Here, we summarize the breeding results and effects on reproductive organs for this study. Detailed results and analyses will be presented elsewhere.

METHODS

Sexually mature, wild-caught male ($n=12$) and female ($n=30$) Norway rats (*Rattus norvegicus*) were live-trapped in Fort Collins, CO, dusted with an insecticide to kill ectoparasites, and transported to the NWRC. Upon arrival, rats were housed individually in cages and provided commercial rat chow and water *ad libitum*. All rats were quarantined for a 3.5-week period to allow any females that were pregnant at the time of capture to deliver litters.

After quarantine, rats were housed in arenas to simulate open breeding conditions. Arenas were outfitted with den boxes with bedding and nesting material, enrichment items, and small structures for the rats to climb. Commercial rat chow, water, and ContraPest® (active or control) were provided *ad libitum*. ContraPest® (active or control) was dispensed from Helland Liquid Dispensers (JT Eaton, Twinsburg, OH) housed inside bait boxes.

Non-pregnant female rats were placed in the rooms 24 hours prior to the male rats. Bait (active or control), food, and water were available immediately upon introduction of the females. Bait consumption was monitored every two to three days and replaced as needed for the first 56 days of the experiment. After 56 days, treatment bait was removed and all rats consumed control bait.

Rat rooms were checked daily to monitor the overall health and well-being of the rats. After 2.5 weeks in the rooms, rats and den boxes in each group were checked daily to monitor for birth of pups. At parturition, pups were removed, counted, sexed, and euthanized. After three months (approximately three breeding cycles), male rats from both rooms were live-trapped and placed into the other group room to simulate more natural breeding. After the fourth breeding round, all rats were trapped out of the rooms, euthanized, weighed, and necropsied.

RESULTS AND DISCUSSION

After three breeding rounds, there was a 95% reduction in pups born to treatment breeding pairs ($n=12$ pups) compared to controls ($n=255$ pups; Table 1). In the final round, 93 pups were born to control females paired with treated males, and 80 pups were born to treated females paired with control males. Necropsy data revealed significant ($P < 0.05$) reduction in testis weight, epididymis weight, and testis volume in treated males. Treated females displayed a statistically significant ($P < 0.05$) decrease in ovarian weight.

Table 1. Reproductive success for wild-caught Norway rats treated with ContraPest® versus controls.

Group	Total Pups	Number of litters	Average Pups per Successful Litter
Control x Control	255	30	8.5 ± 0.46
Treatment x Treatment	12	2	6.0 ± 3.0
Control F x Treatment M	93	14	6.6 ± 0.84
Control M x Treatment F	80	12	6.6 ± 0.98

With the demonstration that ContraPest® is effective at curbing reproduction in wild-caught rats in a controlled cage setting (Witmer et al. 2017), the present experiment was designed to determine whether the same outcome would result in wild-caught rats in an open breeding situation. Females and males repeatedly chose to consume the bait, and this free selection is essential for future field trials where the ability of the bait to reduce wild rat populations will be assessed in agricultural and urban systems. Overall, we conclude that ContraPest® is a highly efficacious fertility control bait that has the potential to induce sustained infertility in the Norway rat with unlimited access to bait.

ACKNOWLEDGEMENTS

This study was funded by SenesTech, Inc., Flagstaff, AZ, through an agreement with the USDA National Wildlife Research Center, Fort Collins, CO. The study was conducted under the NWRC IACUC-approved study protocol QA-2151. We thank the landowners who allowed us to live-trap Norway rats on their properties for the study.

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