A Field Evaluation of the Efficacy of Milorganite® as a Repellent for Non-Venomous Rat Snakes (*Elaphe obsolete*)

GEORGE R. GALLAGHER
Department of Animal Science. Berry College, Mount Berry, GA 30149, USA

McKENZIE WEISSER
Department of Animal Science. Berry College, Mount Berry, GA 30149, USA

DARYON SMITH
Department of Animal Science. Berry College, Mount Berry, GA 30149, USA

DANIELLE CREAMER
Department of Animal Science. Berry College, Mount Berry, GA 30149, USA

ABSTRACT: The objective of this study was to evaluate the efficacy of Milorganite® as a repellent for rat snakes. Milorganite® is the bio solids by-product left from the activated sludge process from the Milwaukee Metropolitan Sewer District. During 3, 7-day release periods, 5-6 mature rat snakes were placed within a 0.1ha plastic fence enclosure intended to impede escape. The enclosure contained natural and artificial hides and water. Snakes were fitted with an externally attached radio transmitter with location of each snake determined 3 times per day by radio telemetry and visual confirmation. During the first 2, 7-day period, with no Milorganite® treatment, snakes were contained within the enclosure for a similar ($p>0.05$) duration of 9.1h±1.8 and 9.4h±1.8 respectively, before escaping. Prior to release of snakes in period 3, a total of 907.2g of Milorganite® was applied by hand in a 20cm width strip along the interior perimeter of the enclosure fence. During period 3, 6 snakes were maintained within the enclosure longer ($p< 0.005$) compared to periods 1 and 2, with an average containment time of 23.5h/day±0.5. Total snake-hours that animals were maintained in the enclosure was higher ($p<0.005$) during the Milorganite® treatment (164.0h±1.4) compared to non-treated period 1 (64.0h±1.8) or period 2 (66.0h±9.0). All snakes remained within the enclosure throughout the 7-day treatment period. One snake died on day 6, post-treatment from unknown causes. Results of this study suggest Milorganite® was effective as a repellent for the rat snake under these experimental conditions.

Key Words Milorganite, radio telemetry, rat snakes, repellent, snake enclosure.

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While the desire to repel snakes from an area is not a new concept, identification of compounds determined effective has been limited. Flattery (1949) tested materials ranging from DDT, rotenone, arsenic, chlordane, nicotine sulfate and various gasses. Extensive testing of home remedies including; moth balls, sulfur, cedar oil, lime, coal tar, creosote, liquid smoke, King snake musk and artificial skunk scent has been documented (San Julian and Woodward 1985). While several of these compounds were lethal, none were reported to be effective as a repellent in either of these studies. Numerous fumigants, pesticides, toxins and natural aromatic oils from woody plants have been tested on brown treesnakes (*Boiga irregularis*), with results ranging from no effect, to classification as an irritant or being lethal (Kraus et al. 2015, Clark and

Shivik 2002, Savarie and Bruggers 1999). Varying results of repellent properties have also been reported for commercial products such as, Liquid Fence and Shoo Snake (Sukumaran et al. 2012). One of the first commercially marketed repellents, Snake-A-Way (7% naphthalene and 28% sulfur) has been found to have limited effectiveness on numerous species of venomous and non-venomous snakes (Moran et al. 2008, Ferraro 1995, Marsh 1993). In a previous study, Milorganite®, the biosolids by-product left from the activated sludge process from the Milwaukee Metropolitan Sewer District, demonstrated significant potential as a repellent for non-venomous snakes (Gallagher et al. 2012).

Numerous compounds tested as deterrents were based on influencing the olfactory senses of snakes. Chemical sensitivity of the olfactory system in snakes is reported to be the most important sense in prey detection, orientation and sexual behavior (Muntean et al. 2009). The tongue itself may increase odor-sampling area and directly transfer contacted chemical to a highly developed vomeronasal system for analysis (Muntean et al. 2009, Parker et al. 2009). Based on gene analysis of olfactory receptors, it was predicted that snakes rely heavily on the olfactory receptor system as a method of odor detection (Byerly et al. 2010). Ferraro (1995) suggested examining repellents or olfactory based compounds based on confinement studies that removed the snake from the natural environment and allow only two choices, failed to give reliable accurate results. While numerous methodologies have evolved to examine repellent properties and snake behavior, most studies rely on relatively small evaluation chambers that exclude the natural environment (e.g., Kraus et al. 2015, Sukumaran et al. 2012, Gallagher et al. 2012, Clark 2007, Clark and Shivik, 2002, Renapurkar et al. 1991). Therefore, the objective of this study was to evaluate the potential of Milorganite® as a repellent for rat snakes (*Elaphe obsolete*) under simulated field conditions, in an outdoor enclosure encompassing a more natural environment.

STUDY AREA

This study was conducted on the 1,215 ha Berry College Wildlife Refuge (BCWR) within the 11,340 ha Berry College campus in northwestern Georgia, USA. The BCWR was within the Ridge and Valley physiographic province with elevations ranging from 172 m to 518 m (Hodler and Schretter 1986). The BCWR was characterized by campus-related buildings and facilities for the 2,100 student body, is interspersed with expansive lawns, hay fields, pastures, woodlots, and larger forested tracts. The site used for this study was characterized as an unimproved pasture at the Berry College Sheep Center. The area was not being used for grazing of domestic sheep during the study conducted, June 23, 2016 – July 28, 2016. The forage consisted predominantly of fescue (*Schedonorus phoenix*), orchard grass (*Dactylis glomerata*), and interspersed with Bermuda grass (*Cynodon spp.).* Forested areas within 200m include various species of pines (*Pinus* spp.), oaks (*Quercus* spp.) and hickories (*Carya* spp.).

METHODS

Construction of a snake enclosure began with a 25cm trench dug in a 30mx30m square (0.1ha) in an unimproved pasture that had timber selectively cut at least two years previously. Wood posts (8.9cm x 8.9cm x 2.0m) were secured on corners and at 15m intervals between each corner at an average height of 128.5cm±0.5 with an inward slope of 17.1^{o} ±0.5. Steel T-posts (2.0m) were erected to a similar height and angle at 4m intervals between wood posts and fitted with plastic insulated caps. Three strands of 17-

gage wire were secured to the top, middle and 10cm above the ground of each post. Plastic sheeting (3.04m x 30.4m x 4mm) was draped over the suspended wires with the bottom 25cm secured within the trench with dirt. All overlapping seams of plastic were secured with polypropylene tape. A single strand of the 17-gage electric wire was attached to the top inside edge of the plastic fence using duct tape. An additional strand of electric polyfence tape was also attached by duct tape to the top of the inside of the plastic fence, and to the plastic 20cm above the ground. A loop (4m) of electric polyfence tape was placed in each of the four corners of the enclosure and attached to both the top electric wire and polytape and the lower section of polyfence tape, energized by a solar powered charger with an output >5000v. In addition to natural hides, 16 artificial hides constructed of 2cm x 61cm x61m plywood were placed in the enclosure with 4 artificial brush hides, and 8 plastic containers to provide water.

Mature wild rat snakes (n=11; 138.1cm± 5.8) were hand captured, placed in 40L secure aquariums and provided water and food. Radio transmitters (Ag392, Biotrack LTD., Wareham, Dorset, UK) were attached externally approximately 25cm cranially to the cloaca, using cyanoacrylate glue and camouflaged duct tape. Each snake was provided a mouse as a food source prior to release and between each release period. During each of three release periods, 5-6 snakes were released into the enclosure typically within 48-hours of capture. The location of each snake was determined using the externally attached radio transmitters and tuned receiver (R-1000, Communications Specialist Inc., Orange, CA), 3x/day for each 7-day period. Snakes that escaped and recaptured were utilized in subsequent releases.

Prior to the second release of snakes, day/night infrared cameras (SN502-4CH; Defender Inc., Cheektowaga, NY) were positioned 10m from each corner of the enclosure, to provide continuous recordings on DVR's. Immediately before the release of snakes in period 3, a total of 907.2g of Milorganite® (226.8g/side) was applied by hand in a 20cm width strip along the interior perimeter of the enclosure fence. Analysis of the duration snakes were maintained within the enclosure was conducted using one-way ANOVA analysis procedures of IBM SPSS 24.0 (SPSS 24.0 2016). This experiment was conducted with the approval of the Berry College Institutional Animal Care and Use Committee and under the Georgia Department of Natural Resources Scientific Collecting Permit.

RESULTS

During the first 2, 7-day release periods, with no Milorganite® treatment, snakes were contained within the enclosure for a similar ($p>.05$) duration of 9.1h±1.8 and 9.4h±1.8 respectively, before escaping. Prior to release of snakes in period 3, a total of 907.2g of Milorganite® was applied by hand in a 20cm width strip along the interior perimeter of the enclosure fence. During period 3, all snakes remained within the enclosure throughout the 7-day treatment period. It should be noted that one snake died within the enclosure on day 6 of the 7-day period. There were no indications of a specific cause of death following a necropsy. Thus, containment was longer ($p< 0.005$) compared to periods 1 and 2, with an average time of 23.5h/day±0.5. Total snake-hours that animals were maintained in the enclosure was higher ($p<0.005$) following Milorganite® treatment (164.0h±1.4) compared to non-treated period 1 (64.0h±1.8) or period 2 (66.0h±9.0). Results of this study suggest Milorganite® continues to provide evidence as a potential repellent for snakes.

DISCUSSION

Anecdotal evidence of the effectiveness of Milorganite®, the biosolids by-product left from the activated sludge process from the Milwaukee Metropolitan Sewer District, as a repellent for numerous species is reported. It has been documented to reduce damage from white-tailed deer to ornamental plants, horticultural and food crops (Gallagher et al. 2007, Stevens et al. 2005). The compound likely elicits its effect through the olfactory system. As indicated by Clark and Shivik (2002), identification of repellents that are effective with minimal toxicological risks to humans and the environment would be ideal. Toxicology reports provided by the manufacturer suggest limited risk to humans, animals or the environment (Milorganite.com).

In a previous study, Milorganite® demonstrated significant potential as a repellent for non-venomous snakes in an indoor testing environment (Gallagher et al. 2012). However, numerous challenges occur when conducting studies that involved confinement and limited choices. Ferraro (1995) indicated that most repellent studies involved removal of snakes from their environment and placing them in an unnatural restricted containment structure. The animals are typically subjected to a treatment or control option that forces the snake to choose an action with only two options failed to give reliable or accurate results.

In the current study, it was attempted to provide a larger, more natural environment complete with natural and artificial hides and sources of water. Construction of a fence intended to contain the animals within the .1ha enclosure was deemed necessary in order to have sufficient numbers of animals to test the treatment.

Maintaining snakes within the fence constructed alone was not successful. Prior to application of Milorganite in period 3, snakes were contained within the enclosure for only 9.1h±1.8 and 9.4h±1.8 post-release, during the first two periods, respectively. While incorporating the use of electrified wire and electric polytape followed recommendations by Perry and coworkers (1998), video evidence indicated snakes used the electrified polytape in the corners to escape the enclosure. This weakness is likely due to insufficient grounding of the snake to receive a shock and not the concept of incorporating electricity as a part of an effective snake fence.

Detection of the externally mounted transmitters was typically <50m. While this range was sufficient to assist in locating snakes within the enclosure, it often was not effective when attempting to locate snakes that escaped the fenced area. During the first two releases of snakes (n=10), animals breeching the fence were frequently recovered. However, four individuals escaping the enclosure and not located using radio telemetry, ranged from 1-21d post-transmitter attachment, (12.3d± 4.7). At the end of the third period, the fence was removed allowing the five remaining snakes with transmitters attached to disperse. Despite a series of extensive search efforts, no snakes could be located or recovered within 12h of the fence removal.

While recovery of externally mounted transmitters occurs with ecdysis, snakes (n=4) shedding their skin and the transmitter prior to the end of the study was also problematic. In this study, transmitters that were recovered as a result of shedding occurred within 6-17d post-attachment (11.7d ±2.4). This effect could be avoided by keeping snakes in a captive environment until ecdysis is complete and then attaching transmitters.

It is recognized that while the enclosure fence was not successful in preventing snakes from leaving the experimental site, its presence likely influenced behavior. Regardless, the fact that all snakes were maintained in the enclosure after treating the interior perimeter of the fence suggests Milorganite® was a significant contributing factor in eliminating escape, thus providing additional evidence as a potential repellent for the rat snake.

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